

nature

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Genetic guidelines: handle with care

PERFECT democracy or perfect dictatorship? That is a question which might well have to be asked now that the *Report of the Working Party on the Practice of Genetic Manipulation* has been published (Cmnd 6600, HMSO, 50p). Its recommendations embody a principle that Britain already knows in respect of its pay policy: that, threatened by a legislative hammer, everyone 'voluntarily' acquiesces in publicly announced guidelines.

The working party divides experiments into four categories (depending on their hazards) and provides a code of practice to cover each category (for details, see page 4). Researchers will submit details of their experiments to a Genetic Manipulation Advisory Group (GMAG), which will vet and categorise the work. They can then decide voluntarily whether or not to abide by the advice of the GMAG and to follow the code of practice.

But at the same time, details of the experiments will have to be submitted to the Health and Safety Commission (HSC), which has turned its eye specifically to all forms of genetic manipulation of micro-organisms, including those which preceded the advent of the new technology for recombinant DNA. The HSC will, through its inspectors, police the work using the existing (and very wide ranging) Health and Safety at Work Act, which it administers. The great advantage of this Act is that it applies to all employers, in industry, public laboratories and universities. This has allowed the working party to avoid the problem being faced in the United States, where guidelines control only research sponsored by the National Institute of Health (NIH) and leave industrial and defence-related work largely untouched.

So unspecific is the legislation, in fact, that it could probably cover any laboratory which carried out any experiment deemed to be hazardous to those who worked there. But the HSC is now considering what special provisions it might need to give it more specific and authoritative control of genetic manipulation experiments. So the Act will be reinforced by a set of regulations, a draft of which has been produced. Their final form will depend on suggestions and recommendations received between now and November. One possibility is that the HSC will adopt the commendably detailed Code of Practice proposed by the working party, thus giving it a basis in law.

The GMAG, which will produce an annual report,

will clearly be a powerful body. To its credit the working party offers the GMAG flexibility by refusing to go into the kind of detailed categorisation of experiments produced by the NIH in its analogous regulations; the preference, plainly, is for learning by experience. In addition the working party has considered not just the medical hazards of genetic manipulation in isolation, but also the potential dangers to plant and animal populations, which are included within the GMAG's scope.

The members of the GMAG, which will be responsible to the Secretary of State for Education and Science, will be announced as soon as possible. The main problem is likely to be the initial bottleneck in dealing with the batch of proposed experiments, some of which have lain dormant for two years. The GMAG is going to have to work speedily to overcome its own inexperience and the growing impatience of those now being encouraged by the working party, who have postponed their most exciting experiments.

Even more of a problem may face the biological safety officer, whom the working party recommends be appointed in every laboratory carrying out genetic manipulation experiments. His task, particularly in the higher-risk laboratories, would be as onerous as it is important. And in the event of any prosecution under the Health and Safety at Work Act, he could not necessarily pass the buck on to the head of department to whom he is responsible. Given this, and the disinclination of most people to control the work of their colleagues, the job of biological safety officer may not prove to be a popular one. Might there not be a case, particularly in those laboratories with a major interest in genetic manipulation, for the appointment of a suitably trained person to that specific and sole role?

There is one slightly worrying aspect about the way the proposals have emerged. It relates to the British distaste for debate in full public view. Thus far, it has certainly been possible to state a view and have it considered by those deliberating the issues—but there has been nothing like the openness afforded in the United States. Greater openness would have produced an equally satisfactory result, and perhaps as quickly. But it would have also have meant greater legitimacy for the final course of action. The effectiveness of the proposals will be a reflection of how well or badly British science manages itself. □



Off the tobacco road

UK cigarette manufacturers have reached a crucial stage in the development of substitute tobacco. **Allan Piper** reports

CIGARETTES containing what have become known as "supplements" could be on the UK market within 6 to 9 months. Rigorous health trials, recommended by the UK Independent Scientific Committee on Smoking and Health, should be completed soon, and three major tobacco companies with British interests—Carreras Rothmans, Gallahers and Imperial Tobacco—are then expected to submit their results to the committee.

The committee is an advisory body to the Department of Health and Social Security (DHSS) set up in 1973 under the chairmanship of the Vice-Chancellor of Birmingham University, Dr R. B. Hunter. If it is satisfied that the findings meet the guidelines it laid down last year, the committee is expected to recommend the licensing of supplements for immediate commercial development in Britain.

One company won't be on the early bandwagon. Courtaulds decided last month to hold back on its development of a tobacco substitute. The decision, however, was merely the commercial judgment of a company temporarily sidestepping an area of heavy pioneering expenditure which is already covered by the others in the field. Once supplements have been broadly sanctioned, of course, Courtaulds can jump straight back into the arena, reasonably certain that the £3 million, which it needs to carry its own product, Tabrelle, through the Hunter tests, is unlikely to disappear down the drain. The company will be up to two years behind those already through the trials. But it can take this course because Tabrelle is essentially similar to the other products being tested by the tobacco companies.

Two types

There are, in fact, only two basic types of supplement. One is made up of largely inorganic materials called inert diluents, the other is based on organic cellulose derivatives. Both use an organic binder. On current medical reckoning—that the cigarette cancer risk stems directly from the tar content of smoke—both kinds of supplement provide a safer alternative to ordinary tobacco. Organically based varieties, such as Tabrelle and the unimaginatively named NSM ('New Smoking Material') give less than

40% of the amount of tar produced by ordinary tobacco. With Cytrel, the main inorganic variety, even that reduction can be improved two-fold. This relative advantage is reinforced further because the organic NSM produces more carbon monoxide, aldehydes and so-called furan derivatives than tobacco, perhaps posing an additional, though as yet undetermined, health threat.

In the event, none of the major tobacco companies in Britain has plumped squarely for one type of supplement rather than the other. Gallahers is maintaining an equal interest in both Cytrel and NSM. And while Carreras Rothman and Imperial Tobacco have moved more positively in one particular direction, respectively favouring Cytrel and NSM, both companies have kept their eyes on the alternative option.

These preferences have resulted in close research links between the retailing tobacco companies and the manufacturing chemical corporations. Carreras Rothman have an arrangement with Celanese, the US makers of Cytrel, while the tie between Imperial Tobacco and ICI has led to the formation of the joint consortium NSM Ltd. planned as an eventual commercial manufacturer and supplier. In Germany, the chemical giant Bayer has just terminated a liaison with Reemstma, following the spectacular failure there last year of two brands containing supplements. As for co-operation between the companies and the Hunter Committee, this is coordinated by the Tobacco Advisory Council and has apparently been effective, in spite of misgivings within the industry about the Committee's strong academic make-up.

Three stages

The Hunter guidelines separate tests into three stages, with an interim check at the end of Stage I. This first stage, already passed safely, included comprehensive studies of smoke chemistry, together with inhalation tests using rats and monkeys over a period of at least six weeks. Stage II involved short-term clinical studies of respiratory irritation in human volunteers. At this stage the tobacco companies were also free under the guidelines to conduct

controlled consumer trials to assess such characteristics as flavour acceptability.

The third stage, now virtually completed, involves the most rigorous tests of all. Studies at this level fall into three categories: carcinogenicity trials, which include the classic mouse skin-painting test; reproduction and teratological studies, designed to assess possible side-effects of smoking supplements; and, finally, life-long inhalation studies on rats and other species, which in Britain has already led to the controversy over the use of baboons and beagles.

In a world context the Hunter guidelines are unique, and most interested corporations outside Britain hope that once their UK competitors have borne the expense of breaking new ground, Hunter Committee approval can be used to ward off future criticism of supplements on respective domestic fronts. The £3 million cost for the tests carry total development costs for the tobacco companies in Britain to around £10 million each over the past five or six years.

When the committee licences the supplements, marketing will begin. For historical tax reasons they are still grouped with additives in the Finance Act, but new legislation to bring them under the Medicine Act is expected to be through parliament within the first couple of months of next year. Without hitches, cigarettes containing up to 25% supplement could thus be on the British market by March 1977.

NSM Ltd already has a large production plant ready at Ardeer in Scotland, a £14 million investment by Imperial Tobacco. Reports indicate that Courtaulds could have Tabrelle in commercial production within a year of the Hunter green light for an additional investment of £10–20 million. And from across the Atlantic, Celanese can be expected to provide all the Cytrel needed in the early stages of UK marketing. Similar products such as the Anglo-American Batflake and Bayer's still mysterious blend can also be expected to emerge rapidly once they have passed through the Hunter tests.

Failure to win licences at the first attempt would mean a major setback, but the search for a safer cigarette will certainly not be abandoned, even though supplements are by no means universally accepted as the most effective way to reduce the smoking risk. Improvements to existing filters, particularly through ventilation, offer another possibility, as does an increased porosity of cigarette papers. The search meanwhile continues for that elusive premium blend of tobacco which gives maximum possible taste with minimum possible tar. □

The OTA on the EPA

Will Lepkowski looks at the US Environmental Protection Agency, the subject of a recent report.

WHEN the Environmental Protection Agency was formed in late 1970, one of the justifications for its creation was that a single agency could take better responsibility for restoring environmental quality than a group of separate fiefdoms. EPA, the argument went, would view the environment as a single, interrelated whole through an administrative structure that was itself correspondingly integrated. Environmentally conscious America heaved a sigh of assurance that at last the quality of life and the preservation of Space-ship Earth were given the policy enthronement they merited.

While the human cell may be the perfect blending of structure and function, EPA decidedly is not. It has faced many reorganisations of its research and development branches, internal controversies, budgetary disappointments and manpower freezes; it has suffered from conflicting Congressional mandates, court setbacks to its enforcement decisions, political shoving matches, and has had its programmes skewered by new energy policies. Little wonder, then, that EPA continues to find the exercise of leadership elusive.

In the past, EPA was buffeted by policy fashion as well as by legitimate currents of change. Until the late 1960s, for example, environmental control was seen as a problem of public health protection, and was thus housed in the Department of Health, Education, and Welfare (HEW). Gradually, however, the emphasis began to shift from defining health problems as guidelines for environmental control to achieving technological solutions. The health problem obviously remained, but Congress decided that HEW was no place for the development of technology or the understanding of complex ecological systems, and EPA was created.

It can be fairly stated that EPA never could boast of a consistent research policy. Hopes ran high during its first two years when systems analysts descended upon headquarters. But systems mythology could only but clash with traditions rising out of wet chemistry and sanitary engineering. Goals seldom squared with realities, especially in environmental regulation where research strategies were usually dictated by crises emerging each week. To be an EPA researcher was to be unhappy. Besides being bureaucratically overarching but arthritic, the agency

was acutely politicised by the Nixon Administration.

Five year plan

Out of the wreckage—but in many cases reflective of it—rises a five-year research and development (R&D) plan that Congress's Office of Technology Assessment (OTA) has just finished analysing for the committee on science and technology. OTA has little good to say of it; indeed, during the assessment process, EPA scrapped the word "plan" and substituted "outlook". It says in essence that EPA's plan is not a plan and as such is no guideline for helping direct long term policies for the country.

"Foremost among the shortcomings in the R&D plan," OTA's report says, "is EPA's failure to indicate a commitment to long range research and, as a corollary, an excessive focus on short term R&D issues related directly to the enforcement and/or achievement of EPA's current regulations. Accordingly," it goes on,

... the Plan emphasises the development and demonstration of control technologies. In many cases, however, the larger problems involve social, economic and institutional patterns which not only impede technical solutions but which require nontechnical approaches. To develop effective overall environmental management strategies will require more systematic and sustained socioeconomic research efforts than those specified in the Plan. An added R&D emphasis on long range environmental concerns and a more responsive role to its line of responsibility as coordinator of Federal environmental R&D would do much to enhance EPA's effectiveness and credibility.

The US is obviously already environmentally conscious. Whether it is environmentally cleaner is another question. The recent outbreak of the so-called "legionnaires disease" in Philadelphia appears to be caused by a toxic metal whose origins and precise identity are still under investigation. Evidence points to nickel carbonyl from microcapsulised paper that requires no carbon paper for the typing of copies. Whatever the cause, the point is that toxic chemicals are everywhere in the environment, and EPA must always play a "catch-up" role.

But anticipation is the name of the environmental game in its ideal, and EPA's record as an anticipatory research agency is a sorry one. Yet, anticipatory research is a sorry subject given the complexities of industrialisation and ecosystems. The obvious alter-

native is to require industry to stop producing anything toxic to begin with. EPA would administer the pending toxic substances control Act which would do just that, but Congress seems hesitant to pass a law so distasteful to industry. It has been "pending" for almost a decade. If passed, however, EPA would become in theory much like the Food and Drug Administration with its main regulatory goal being prevention rather than reaction. Wondering what to react to has always produced bureaucratic psychosis.

EPA's problems over the last few years have been money, manpower and morale. The EPA report doesn't appraise the morale problem because the charge was only to assess the Plan itself. But the panels which gathered to make the critique were composed of individuals familiar with the agency's internal problems and the moribund psychology of the agency. That issue wasn't discussed, despite its centrality. The research arm of the agency is plainly dejected. Individual scientists know what needs to be done to begin anticipating the environmental future, but they know too that doing it would require ten times the present research budget of \$250 million.

Questionable help

Thinking about environmental futures is often a pathway to dejection, especially considering the fact that industrialisation of the less developed nations entails no pollution control to speak of. And oceanographers have long since despaired to any effective pollution control policy for the oceans.

How much of a help OTA's critique of the plan will be is questionable. It says it isn't a plan. It says the main issues are socioeconomic and EPA hardly knows how to ask the right questions in such a fuzzy area of parasitology. It says EPA knows next to nothing about the long term effects of pollution. It says EPA has a weak health research capability. It is a litany of deficiencies. The sorry fact is that the same criticisms were made of environmental research 15 years ago.

The findings have long since been seen and mulled over by the House science and technology committee. But the practical and philosophical deficiencies pointed out in the OTA report transcend solution without profound changes in consciousness among those who make budgets and plan strategies. And, of course, the entire milieu within EPA would change once Congress passes the toxic substances bill. But that would mean further growth in the regulatory bureaucracy and, of course, larger costs of doing business. The present Administration opposes both. □

BRITAIN

Genetic manipulation: guidelines out

Eleanor Lawrence studies the report on genetic manipulation experiments and the consultative document on regulations to control them.

GENETIC manipulation experiments involving artificially prepared recombinant DNA elements are to go ahead in the United Kingdom under certain specified conditions. Those intending to do such experiments must notify the Health and Safety Commission (HSC), an arm of the Department of Employment, and will also notify an independent Genetic Manipulation Advisory Group (GMAG) which will advise them of the conditions of containment required. The HSC is seeking powers to enforce the conditions recommended by the GMAG, and has put forward a set of draft regulations.

The voluntary code of practice and the proposed establishment of a national Genetic Manipulation Advisory Group are the main recommendations of the long-awaited report of the Williams Working Party on the Practice of Genetic Manipulation, published last week with government approval*. The aim of the technical guidelines and the machinery to put them into practice is to protect workers and the general public from the possible hazards presented by bacteria into which foreign genes have been transplanted by novel techniques developed several years ago in the United States.

These techniques enable DNA from another source (be it human, animal, plant, virus or an unrelated bacterium) to be spliced on to DNA from a phage or plasmid vector and introduced into a bacterium. The fear is that bacteria modified in this way (especially the common gut bacterium *E. coli*, a favourite of the genetic engineers) might present unpredictable dangers because of the new genes they possess, especially if the inserted DNA is not properly characterised or is known to contain potentially dangerous genes. The dangers could apply not only to the human and animal populations; harmless soil or plant bacteria into which genes from plant disease agents have been introduced could possibly pose a threat to crops.

But the advantages, both scientific and practical, which may eventually accrue from these new techniques are so great that there has been enormous pressure for the work to go on. Not

only does it make it possible to clone particular genes and thus get to grips with many tricky problems in genetics, but specially engineered bacteria might eventually be induced to express the inserted genes and act as custom-built mini-factories making a valuable product quickly and cheaply.

Code of practice

The Williams working party was set up in response to recommendations contained in the Ashby Report which, in 1975, first considered the implications of the new genetic engineering techniques. Its brief was to establish a code of practice for conducting these experiments as safely as possible, and to define the role of the central advisory group which Ashby proposed. This, it was envisaged, would be responsible for ensuring that workers intending to perform genetic manipulation experiments were fully conversant with the safe microbiological techniques required.

Addressing the issue of matching particular experiments to the appropriate containment level, the working party has distinguished some 20 separate types of genetic manipulation experiment (see table) which they have then assigned to one of four levels of physical containment.

● **Category I:** Experiments must be carried out in conditions suitable for containing common human pathogens. (Because the introduction of alien DNA into a bacterium might have unforeseen consequences, the working party recommends that these precautions must be taken even when the source DNA, the vector and the host bacterium are all perfectly harmless in themselves).

● **Category II:** As I, but in addition the laboratory must be sited away from areas used by the general public and must have controlled air flow and an exhaust-protective cabinet for aerosol-producing operations.

● **Category III:** As II, but in addition access to the laboratory must be through an airlock only (which should contain washing facilities), all effluent from the laboratory must be decontaminated, and there should be an autoclave inside the laboratory.

● **Category IV:** Conditions should be equivalent to those already recommended in the Report of the Working Party on the Laboratory Use of Dangerous Pathogens for the most dangerous human and animal pathogens. (There are only a few such laboratories

in existence at present, for example those at the Center for Disease Control at Atlanta, Georgia, and at the Microbiological Research Establishment at Porton Down in the UK).

Genetic manipulation experiments can also be made safer using host bacteria and vectors which have been disabled so that they cannot grow outside a particular laboratory environment. Hosts can be crippled by introducing a requirement for some particular growth factor not found in the human or animal body, and disabled vectors which can only infect a special laboratory strain of host can be made.

The working party acknowledges the value of disabled organisms by assigning a lower containment level to experiments in which they are used, and recommends that research to develop new and better disabled strains be encouraged and the strains be made freely available.

Recommendations for implementation

The working party's recommendations are also concerned with the machinery needed to ensure that the code of practice is in fact followed. It recommends supervision at two levels, nationally

What is suggested ?

The Williams report recommends that:

1. experiments in genetic manipulation, conducted in appropriate conditions of physical and biological containment, should be encouraged.
2. further work should be done on the development and characterisation of disabled organisms and that any which are developed should be made freely available to all workers in the field.
3. no genetic manipulation experiment should be undertaken in containment conditions less stringent than those used for work with common pathogens.
4. the code of practice (in Appendix 11) should be adopted as a basis for the conduct of these experiments.
5. every laboratory conducting these experiments should have a safety committee and a Biological Safety Officer.
6. appropriate training should be made available and be required for all research workers, technicians and biological safety officers in genetic manipulation laboratories.
7. a Genetic Manipulation Advisory Group (GMAG) should be established to advise on appropriate precautions for the conduct of these experiments.
8. the GMAG should be separate from the Dangerous Pathogens Advisory Group (DPAG) although there should be liaison between the two groups.
9. a system of voluntary control should be established as quickly as possible.
10. regulations should be made under the Health and Safety at Work Act to require laboratories to submit experimental protocols to the GMAG.

* *Report of the Working Party on the Practice of Genetic Manipulation* (Cmd. 6600, HMSO, 50p).

through the GMAG and, on the laboratory floor, in the person of a Biological Safety Officer, to be appointed in every laboratory in which genetic manipulation experiments are being carried out.

The Williams working party sees the central advisory group as crucial to the success of any control measures. One of its main functions will be to advise on the category into which a particular experiment should fall and on whether the laboratory is competent to carry it out. To do this the GMAG will need to maintain records of the facilities available in different laboratories; it should in time establish a register of approved laboratories. It should also review experimental protocols regularly and modify the code of practice in the light of new developments in biological and physical containment, and advise on training. The GMAG will be set up in the near future with a secretariat within the MRC, who will be respon-

sible to the Secretary of State for Education and Science.

The biological safety officer will be the key person in the enforcement of correct procedures at the individual laboratory level. It will be his job to familiarise himself with the required techniques, and check that experiments are being carried out correctly; he will be responsible for training staff, investigating accidents and maintaining the security of the laboratory.

Recognising the "restraint already shown by the scientific community" since the voluntary moratorium on this work called for by American scientists two years ago, the working party now recommends that certain experiments which they consider do not present a serious hazard (Categories I and II, see table) should go ahead immediately, subject to notification to the GMAG. Experiments which fall into categories III and IV should not proceed until proposals have been

approved by the GMAG.

In effect, the working party's technical guidelines are very similar to those published in July by the US National Institutes of Health, though there are important differences between the American and British approach. The US code of practice applies only to work carried out with NIH grants; the UK recommendations will apply to university government and industrial laboratories. The working party has also deliberately avoided the precise assignment of experiments with named organisms to particular categories.

Statutory control

The Working Party recommends that in the first instance the controls should be voluntarily imposed. But the draft regulations proposed last week by the Health and Safety Commission would provide some measure of statutory control without special legislation having to be passed.

The Health and Safety Commission is a government agency under the aegis of the Department of Employment set up to administer the Health and Safety at Work Act. This Act provides very wide powers to bring in regulations covering any work which might pose a hazard to the health and safety of work people and/or the general public.

The draft regulations, which are now being circulated as a consultative document to interested parties for comment, provide for compulsory notification to the Health and Safety Executive of all experiments which are "intended to alter, or likely to alter, the genetic constitution of any micro-organism . . ." As it stands this blanket definition would cover practically all bacterial genetics experiments, and the Executive is asking the scientific community to recommend which conventional types of experiment should be exempted.

In addition the Executive wants to be able to enforce the precautions recommended by the GMAG through the Executive's Inspectors. They can already enforce general measures taken to avoid danger to health, but the Commission is inviting comments from any interested party on the need to include more specific provision for ensuring that the GMAG's advice is taken.

The ultimate sanction for researchers remains the withholding of a grant, however, and the working party recommends that, legal enforcement or no, government departments, research councils and other grant-giving bodies make grants conditional upon investigators following the advice of the GMAG. The working party also hopes that scientific papers will include a statement of both the physical and biological containment procedures used. □

Suggested categorisations for some typical experiments

Source of nucleic acid	Specification of nucleic acid sequence	Vector/Host System	Category
<i>Mammals</i>	Random	Phage or plasmid/bacteria, not disabled	IV
	Random	Phage or plasmid/bacteria, disabled	III
	Purified*	Phage or plasmid/bacteria, not disabled	III
	Purified*	Phage or plasmid/bacteria, disabled	II
<i>Amphibians and reptiles</i>	Random	Phage or plasmid/bacteria, not disabled	III
	Random	Phage or plasmid/bacteria, disabled	II
	Purified*	Phage or plasmid/bacteria, not disabled	II
	Purified*	Phage or plasmid/bacteria, disabled	I
<i>Plants and invertebrates and lower eukaryotes</i>	Random	Phage or plasmid/bacteria, not disabled	II
	Random	Phage or plasmid/bacteria, disabled	I
	Purified*	Phage or plasmid/bacteria, not disabled	I
<i>Mammals Amphibians and reptiles Birds</i>	Random	Virus capable of infecting man or growing in tissue culture cells	IV
	Purified*	Virus capable of infecting man or growing in tissue culture cells	III
<i>Viruses pathogenic to vertebrates</i>	Random	Phage or plasmid/bacteria, disabled	IV
	Purified*	Phage or plasmid/bacteria, disabled	III
<i>Animal viruses, non-pathogenic to man</i>	Random	Phage or plasmid/bacteria, disabled	II
<i>Bacteria specifying toxins virulent to man</i>	Random	Phage or plasmid/bacteria, disabled	IV
<i>Plant pathogenic bacteria</i>	Random	Phage or plasmid/bacteria, not disabled	II
<i>Plant viruses</i>	Random	Phage or plasmid/bacteria, not disabled	II
<i>Bacteria or fungi non-pathogenic to man, animals or plants</i>	Random	Phage or plasmid/bacteria, not disabled	I

* The term "purified" means fractions with little chance of including any unrecognised extraneous sequences. It is of course possible to have sequences selected because of their pathogenicity and these would raise the level of containment required.

Source: Report of the Working Party on the Practice of Genetic Manipulation (Cmd. 6600, HMSO).

FRANCE

Musical chairs in science policy

As talk of changes at the highest levels of French government grew last month, the country's directors of research were already a few steps ahead. A Correspondent reports

THE directors of French scientific and technical research have recently been involuntary participants in a game of musical chairs organised by the government. First, the president of the Centre National d'Etudes Spatiales (CNES), Professor Maurice Lévy, was dismissed by the Minister of Industry and Research, M Michel d'Ornano, for making concessions to striking staff that his assistant had refused to grant in his absence. He has yet to find another post. On the same day, Professor Curien, head of the Department of Scientific and Technical Research (DGRST), replaced Professor Lévy. The move, from a department which coordinates and implements all research policies (except military projects) to a directly operational post, has drawn much comment.

A few days later, Professor Curien, who was director of the Centre National de la Recherche Scientifique (CNRS) until 1973, was replaced at the head of the DGRST by Professor Grégory, who had previously taken his place at the CNRS. Then, to complete these acrobatics, which incidentally only involved physicists, the director of physics at the CNRS, Professor Chabbal, became director-general of the CNRS at the beginning of August.

The government—and in particular M d'Ornano—has shown in this series of decisions an unexpected authority and haste at a time when the credibility of those in power is constantly weakening. The circumstances of the CNES affair are fairly clear. The shrewd and effective Professor Lévy was forced to leave because of the Minister's change of mood—a change which neither his colleagues nor his advisers, who saw the opportunity to bring about other changes, sought to influence. A review of the allocation of the space budget between the programmes of the European Space Agency and the French programme had been due since European agreement on the level of the Agency's activities. The new allocation brought about some changes in the CNES budget which were to make some of its staff redundant, particularly at Toulouse.

With France wanting to guarantee Europe's autonomy regarding applications satellites, especially telecommuni-

cations (the last breath of the great Gaullist doctrine of independence), the CNES gave priority to completing the launcher Ariane. (The Ariane project's director, Yves Sillard, became director of the CNES and assistant to the President a few weeks ago.) Under the threat of redundancies, CNES workers went on strike. The CNES directors were unwilling to give in to the strikers' demands, and maintained their position for several days, only to be overruled in the end by their president, who took the side of the employees. The government, however, intervened.

The causes of the stir this provoked in the research policy establishment are more complex. Two main characteristics differentiate research in France from research in other countries. The arrangement in the universities, where there is an even split between research and teaching, is unsatisfactory; information about what is going on does not permit either good financial management or national coordination. In accordance with the recommendations of the VIIIth Plan, the Secretary of State for the Universities, who is responsible for university research and the CNRS, has created a "Research Mission" directed by Professor Denisse, who was Professor Lévy's predecessor as president of the CNES. The purpose of this mission is to re-organise university research.

The other characteristic of French research is the endemic weakness of industrial and technical research. French industry does not spend enough money on research, as M d'Ornano recently pointed out in parliament. Moreover, public funds normally allocated to industrial research are often put to other uses financing industry. The DGRST, which is for the most part made up of specialists in basic research and is directed by a laboratory physicist, often has great difficulty understanding industrial problems. This results in disagreements with other administrative departments concerned with industry, technology and financial management.

Thus the DGRST often ends up giving its funds (FF640 million in 1976) to the same laboratories. In an attempt to resolve this problem M d'Ornano asked the fiery director of the Centre National d'Etudes des Telecommunications (CNET) for a report on industrial research last April. One of the conclusions of the report was that a sub-department of industrial research and technology should be set up, linked to the Department of Research.



Michel d'Ornano

This proposal was strongly attacked by the DGRST: by its staff on the one hand who, like any other body, do not like the idea of being restructured by a logic they do not understand; and on the other hand by the director, Professor Curien, who distrusts dyarchies. The director was therefore moved to the CNRS. He was replaced by Bernard Grégory, who is also a former director of CERN. The Minister meanwhile stated that a technological representative will be nominated.

The proposal was also strongly attacked initially by another department, the Department of Mines. Although not implied by its title, this department is concerned with industrial research. It is the fiefdom of "mining engineers", a technocratic elite formed by the greatest of the "Grandes Ecoles", the Ecole Polytechnique. The mining engineers have an undying sense of unity: some hold high positions in the Administration, others are responsible for a large number of French industrial enterprises.

With these connections and the technical control of enterprises and industrial products that have by law fallen to it, the Department of Mines has developed a very different concept of industrial research and development from classical French practice since 1958. It is interested in the traditional industries; it tries to encourage collective research, common to all firms in the same sector; it checks that each project follows the objectives of the government (in conservation of energy, improvement and quality of products, working conditions and so on) through a complex series of committees. In short, it can take action effectively on scientific and technical information, industrial ownership, standardisation—and on funds.

If the future technological representative does not share its philosophy, there will be serious conflicts. And the stakes are high, especially for those enterprises which draw extensively on the research budget. □

IN BRIEF

EEC uranium scheme

Last week's announcement by the EEC Energy Directorate of a £400,000 scheme to support uranium prospecting within the EEC member countries came just two months after the report from Euratom forecasting a shortfall in uranium supplies for Europe from the end of the decade. Euratom expressed concern that there was no coverage either by long term contracts or from known reserves of Community producers, and urged an acceleration in exploration and development. The

Energy Directorate says it hopes the new project will discover new uranium sources to secure the EEC's long term supply.

No postgraduate change

The UK Department of Education and Science has declared in a White Paper (Cmnd 6611, HMSO, 28p) that it does not intend to restructure the arrangements for supporting postgraduate education in Britain. It was responding, after a delay of 2½ years, to strident criticisms of the system

voiced by the House of Commons Expenditure Committee. The committee's main contention was that postgraduate education should be shaped chiefly by the needs of the country rather than by the demands of potential students. The DES, while agreeing in broad principle, says that bodies like the research councils are already sufficiently responsive to these needs. The DES also rejects the idea that postgraduate grants should be replaced by any kind of loan, because the short term savings in government expenditure would not be sufficient.

In theory, the more data a scientist is able to collect, the more accurate his work should become. On the other hand, a great deal of information is expensively collected and then never properly used. Sometimes this is a symptom of oversupported research; a scientist who has to do his own field or laboratory work is generally careful how he uses his time, but if he can deploy a squad of assistants he may ask them to make many more observations just in case these may, eventually, prove valuable. And very occasionally they do. But most of the results of even the best planned research remain untouched in dusty archives or even end up in the waste paper basket, although some work is written up and submitted for publication before sufficient information has been collected. It is not easy to decide when is the right time to stop.

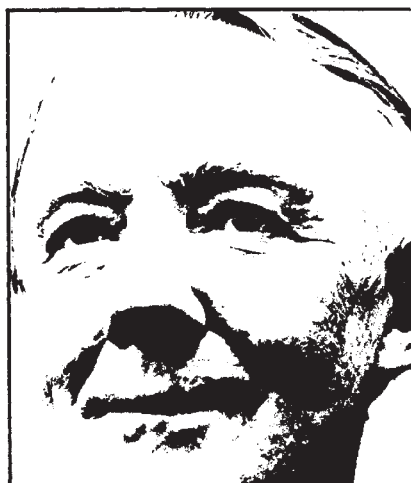
An even more difficult task for the research worker is to decide just what data should be collected. If, to obtain a grant or a fellowship, he has to draw up a detailed programme in advance, he is unlikely to make the same decisions as he would have made after he had become immersed in his problem, particularly if he were personally involved in the details of the work and did not delegate to assistants. It is therefore essential that all research projects should have some provision for review and redeployment at frequent intervals.

But where a team of investigators is involved, such changes of direction may be difficult, as all members may not agree on just what changes are desirable. Even the lone worker may be inhibited from modifying his programme when he realises that this is necessary, in case he upsets the members of some committee to which he reports. Committee members react in this way, and castigate those who suggest that their original submission was faulty, perhaps because they themselves feel guilty for not spotting

the faults earlier.

We may collect too many or too few data, and we may also amass figures which do not provide the information that was intended. This is unfortunately too common an occurrence, and one which may long

Testing rainwater



KENNETH MELLANBY

go unrecognised, as has recently been demonstrated in work on air pollution. Many people are studying problems which involve the contributions to plants and the soil made by the various chemicals contained in rain. Perhaps the best known is that of "acid rain" in Scandinavia, allegedly caused by pollutants, particularly SO₂, released into the air by British and European industry and carried north by the prevailing winds. Other work concerns the growth of crops and trees in polluted and clean localities. Unfortunately few of those involved are making systematic analyses showing exactly what substances are being brought down in the rain.

To many, including some of the investigators, this will appear to be a surprising statement, for numerous

people in many countries are analysing rainwater samples by the thousand, with increasingly accurate methods and more and more sophisticated equipment. There is little reason to doubt the accuracy of their measurements. Unfortunately they are not, for the most part, studying rain, but the liquid which they collect in their rain-gauges. This is something quite different, for it contains not only the chemicals which took part in the formation of raindrops, and those which were collected by the drops as they fell through the air, but also those which had been deposited, since the last shower, on the collecting funnel of the rain-gauge. The dry deposition on the gauge is generally greater, sometimes many times greater, than the quantity of the same chemical in the rain. If this is not realised, and measured, errors of a magnitude seldom experienced in research may arise.

Yet there is no excuse for this possible error. There is a little published information, available for some years, showing how much rainwater may differ from rain-gauge water. We cannot yet say exactly what the dry deposition of materials on any field, crop or forest will be, but there is much elegant work attempting these evaluations, and it is already clear that it greatly exceeds that in the rain, or even that in the already-enriched rain-gauge water.

Those who have spent time and effort analysing rain-gauge water, thinking they were analysing rain, need not despair. Their data are far from useless—they only fail to give the information expected. But this is a common phenomenon in science, and is one of the reasons to be suspicious of too highly organised investigations, particularly those commissioned by customers who think they can guarantee that the new information they seek can always be provided if only the cash is made available.

news and views

Stratified waters hold the key to the past

from M. Whitfield

THE history of the oceans and the atmosphere is, so far as we can tell, a story of the progressive oxidation of the primordial anoxic environment (F. T. Mackenzie, *Chemical Oceanography*, Riley, J. P. and Skirrow, G., eds, 1, ch. 5; 1975). Reducing conditions on the primitive Earth were an essential prerequisite for the formation of organic compounds from inorganic ingredients by a biological process. Oxygen was initially generated by photochemical processes in the atmosphere but large scale oxidation probably did not occur until photosynthetic blue-green algae began releasing free oxygen into the oceans some 3 billion years ago.

Over large areas of the present-day ocean, a permanent density discontinuity (pycnocline) arises as a consequence of the latitudinal variation in the intensity of incident radiation from the Sun which warms the tropical and subtropical surface waters and drives the cold polar waters into the ocean depths. This stratification stabilises the oceanic water column and restricts mixing between water in the sunlit euphotic zone and water from the abyssal depths. It is likely that the pycnocline has been an important feature of the structure of the oceans throughout most of their history (P. K. Weyl, *Science*, **161**, 158; 1968). After the development of photosynthetic cells the pycnocline would also mark the boundary between oxygenated and anoxic water and the resulting stratification would accelerate the build-up of oxygen in the upper layer and enhance the evasion of oxygen into the atmosphere. According to the Berkner-Marshall hypothesis (L. V. Berkner and L. C. Marshall, *J. Atmos. Sci.*, **22**, 225; 1965) the subsequent build-up of atmospheric oxygen played a crucial part in the development of higher life forms since it promoted the switch-over from a fermentative to a respiratory metabolism which was essential for the development of the nucleated eukaryotic cells that are the essential building blocks of all multi-

cellular organisms. The concomitant strengthening of the ozone layer provided a progressively more effective shield against ultraviolet radiation which made possible the colonisation of the energy-rich surface waters of the ocean and eventually the colonisation of the land. The interplay between processes that generate oxygen and processes that consume oxygen in stratified waters is therefore of central importance to any attempts to understand interactions between evolving life forms and their environment.

Pictures of a stratified Precambrian ocean previously developed to explain evolutionary processes (D. C. Rhoads, and J. W. Morse, *Lethaia*, **4**, 413; 1971) or geological phenomena (H. D. Holland, *Econ. Geol.*, **68**, 1169; 1973) have postulated a rather stable, layered system undergoing gradual oxidation over the past 2 or 3 billion years. E. T. Degens and P. Stoffers in this issue of *Nature* (page 22) relating the evolution of the early oceans to the recent history of present-day stratified waters suggest a more variable and dynamic history.

In strongly stratified systems the upper, oxygenated layer is relatively alkaline (pH 8 to 9), the levels of the major nutrients (nitrate, phosphate, silicate) are kept low by the incessant biological activity and carbonate minerals can be precipitated by inorganic or biological processes. The lower layer, by contrast, is more acid (pH 6.5 to 7) and contains hydrogen sulphide and abundant nutrients (ammonia, phosphate, silicate) and free carbon dioxide resulting from the decay of organic matter drifting down from the surface. This layer is also enriched in metals such as iron, manganese and nickel that are soluble in the reduced form. In the lower layer the variety of life forms is restricted to members of the sulphur biome (T. M. Fenchel and R. J. Riedl, *Mar. Biol.*, **7**, 255; 1970) probably the oldest ecosystem on earth.

The stability of the stratified condition depends on the dynamic balance

maintained between the water recirculation (recharging oxygen) and the degradation of organic matter (consuming oxygen). A rapid change in the depth of the pycnocline will cause a hiatus in the water chemistry which will leave its record in the sediments. A rapid downward movement will cause dramatic precipitations of iron, manganese and carbonate minerals and the corresponding injection of nutrients will stimulate plant growth in the oxidised layer. This growth will in turn increase the oxygen demand below the pycnocline and speed up the re-establishment of anoxic conditions. If the pycnocline now moves upwards some of the precipitated minerals (particularly the carbonates) will be redissolved and the incursion of hydrogen sulphide will ensure a massive extinction of bottom-living (benthic) organisms. The banded sediments of the Black Sea and the East African rift lakes observed by Degens and Stoffers, with the precipitates forming discrete layers rather than being dispersed amongst the regular detritus, suggests that the depth of the oxygenated layer has oscillated widely in response to climatic variations and to tectonic and erosional forces throughout the Holocene and Pleistocene. The pattern of variation is strikingly similar in the various locations and, furthermore bears a much closer resemblance to Cambrian and Precambrian sedimentary deposits than do modern oceanic sediments. These similarities suggest that the trend towards an oxygenated ocean might have suffered many reversals and that the details of these reversals are held in the sedimentary record, awaiting interpretation.

The oscillating pycnocline would certainly provide a particularly elegant explanation for the widespread banded-iron formations (rhythmic sequences of iron-rich deposits interleaved with iron-poor cherts) that first appear in the sedimentary record 3 billion years ago and disappear fairly abruptly 1.2 billion years later. More dramatic upheavals of the system could also be engineered

to explain the periodic ecosystem collapses that have occurred at intervals of roughly 150 million years since Cambrian times (H. Tappan, *Palaeogeogr., Palaeoclimatol. Palaeoecol.*, **4**, 187; 1968). The analysis by Degens and Stoffers, particularly of deep sediment cores taken from the Black Sea by the Glomar Challenger, will enable such hypotheses to be tested quantitatively and should allow more definite statements to be made about the stresses that accompanied shifts of emphasis in the evolutionary process in the oceans.

These reminders of instability may also contain a word of warning. Natural control mechanisms are fairly

delicately balanced and they often work by a process of overshoot and damping rather than by rigorous control within fine limits. As the Baltic countries are finding to their cost, increased oxygen demand resulting from industrial effluent can quickly generate oxygen-deficient bottom water in an enclosed sea. Similar problems have occurred in the Great Lakes of North America and have recently been reported in Lake Baikal. Furthermore, artificial upwellings proposed both as a source of power and as a means for stimulating planktonic growth in the surface waters of the oceans may also generate oxygen-deficient bottom water unless they are located with care.

Maintenance and evolution of repeated genes in eukaryotes

from D. Glover

THE genes for 5S RNA and ribosomal RNA are highly repetitive and occur as tandemly repeated clusters within eukaryotic genomes. These genes are separated by so-called spacer sequences which are not transcribed. Two main theories have been proposed to explain the mechanism by which tandemly arranged sequences are kept relatively homogeneous and how the genes and their spacers have evolved together. At the one extreme, are the sudden correction mechanisms such as Callan's "Master-Slave" hypothesis in which many genes are simultaneously corrected against a master template. At the other extreme, gradual correction mechanisms have been proposed which involve unequal crossing-over between homologous chromatids in such a way that variants could be spread or eliminated from tandem genes. The spacers of the genes for 5S DNA and rDNA in *Xenopus laevis* have now been subjected to elegant molecular analysis and this has cast some light on these problems.

The 5S DNA of *X. laevis* has an AT-rich spacer which consists largely of repeated units of simple sequence DNA (Brownlee *et al.*, *J. molec. Biol.*, **89**, 703; 1974). Carroll and Brown (*Cell*, **7**, 467; 1976) have recently described evidence which indicates that there is considerable heterogeneity with respect to length in this spacer sequence. Cleavage with the restriction endonuclease *HindIII* occurs at one site in each 5S gene and generates a series of closely spaced bands each of approximately 700 base pairs. These bands differ in length by regular increments of 14 base pairs which is the length of the simple sequence unit described by Brownlee *et al.* Carroll and

Brown went on to make partial *HindIII* digests of gradient purified 5S DNA, and have cloned these fragments. Each cloned DNA contains up to five of the 5S genes within a DNA plasmid of *E. coli* (*Cell*, **7**, 477; 1976). Denaturation mapping of these cloned 5S genes indicates that adjacent genes can be heterogeneous in length. It is unlikely that such arrangements could be found if sudden correction mechanisms were operating during evolution and Carroll and Brown come down strongly in favour of a mechanism of unequal crossing over between the AT-rich spacers in which the sub-repeats are essentially homologous.

Wellauer *et al.* (*J. molec. Biol.*, **105**, 461; 1976) have now made a similar analysis of the ribosomal genes from *Xenopus laevis*. There are two classes of *EcoRI* fragments which can be excised from the gene unit. One of these is homogeneous for length whereas the other class of fragment is heterogeneous for length and contains non-transcribed spacer sequences. Four representatives of this latter class have been cloned within *E. coli* and then characterised by homoduplex and heteroduplex mapping. This has revealed two regions within the non-transcribed spacer which vary in length and seem also to consist of repetitious simple sequences, the subunit of which is shorter than 50 base pairs in length. In *X. laevis* the ribosomal genes of oocytes undergo substantial amplification, a process which is thought to occur by a rolling circle mechanism of DNA replication (Hourcade *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2926; 1973; Rochemaix *et al.*, *J. molec. Biol.*, **87**, 473; 1976). If this is so, then one would predict that adjacent rDNA genes from oocytes

would be more homogeneous for length than adjacent rDNA genes from somatic tissue. Wellauer *et al.* (*J. molec. Biol.*, **105**, 487; 1976) have examined heteroduplex structures formed between either long single-strand chromosomal or amplified rDNA and a cloned segment of rDNA and have found that this is indeed the case. In a third paper, the same group has found that not only is there heterogeneity in spacer lengths, but also that the pattern of spacer lengths is significantly different from one animal to another so that it can be followed as a genetic marker (Reeder *et al.*, *J. molec. Biol.*, **105**, 507; 1976). The patterns of spacer heterogeneity are stably inherited from one generation to the next and only two animals out of 50 showed changes in their spacer patterns. The mechanism by which these two changes arose remains an open question.

The tandemly repeated gene clusters of *Drosophila melanogaster* are also producing their share of surprises. The nucleolus organisers of *D. melanogaster* are found on both the X and the Y chromosome. In wild-type males there is apparently no meiotic recombination and so the two nucleolus organisers are genetically isolated from one another on non-homologous chromosomes. The rRNA transcribed from either the X or the Y seems to be identical however. A single ribosomal gene unit from this organism has been described which from the evidence of DNA reassociation kinetics also contains about 3,000 base pairs of internally repetitious simple sequence. There are two major size classes of rDNA, one in which the repeating gene unit is 17 Kbases in length and the other in which the repeating gene unit is 11–12 Kb in length (Glover *et al.*, *Cell*, **5**, 149; 1975). Tartof and Dawid (on page 27 of this issue of *Nature*) have examined the distribution of these two units on the X and Y nucleolus organisers and find that whereas the 11–12 Kb unit is present on both the X and Y chromosomes, the 17 Kb unit is found only on the X. This suggests that indeed recombination is not occurring between the X and Y rDNAs, unless there is some specific mechanism for eliminating the 17 Kb units from the Y chromosome.

The mechanism whereby the identity of the rDNA sequences on the X and Y chromosomes is maintained is obscure, and indeed it will be interesting to have a comparison of spacer sequences on the 12 Kb and 17 Kb units which have been cloned in *E. coli*. The mechanism of the maintenance and co-evolution of these tandemly repeating genes may turn out to be even more complicated than the experiments with *Xenopus* 5S DNA and rDNA suggest.

T cell recognition at Cold Spring Harbor

from Martin Raff

The XLI Cold Spring Harbor Symposium on Quantitative Biology, entitled *Origins of Lymphocyte Diversity*, was held on June 1-8, 1976.

SINCE the symposium covered most of present-day immunology this is an attempt to cover just one area—how T cells recognise antigen. In my view, it was recent progress in this knotty area of immunology that provided much of the excitement at the meeting.

While it is clear that membrane-bound immunoglobulin (Ig) molecules serve as receptors for antigen on B lymphocytes, the nature of antigen-specific receptors on T cells has been elusive and, for the most part, unproductively controversial. Now, at last, half of the problem seems to be resolved, with the demonstration that at least one receptor on T cells is a new class of Ig heavy(H) chain, which may come to be known as "tau" (τ). The strongest evidence for this was provided by H. Binz and H. Wigzell (Uppsala University), who, following the lead of Ramseir and Lindenmann¹, have approached the problem using antibodies directed against antigenic determinants associated with the antigen-binding sites of Ig molecules—so-called idiotype determinants.

They had previously shown that anti-idiotypic antibodies (raised in (Lewis $\times$ BN) F₁ rats against Lewis anti-BN alloantibodies, or against Lewis T lymphocytes) react with both T and B cells of Lewis rats which bear receptors specific for the major histocompatibility complex (MHC) antigens of BN rats². Thus, T lymphocytes seem to express some of the same idiotypes as conventional antibodies—a notion supported by similar findings obtained independently by others, in rats³ and mice⁴. Binz and Wigzell have now shown that whereas the idiotypic-bearing (and BN-antigen-specific) receptors on Lewis B cells are 8S IgM molecules, those produced by T cells are polypeptide chains of molecular weight approximately 75,000 daltons, which may exist as dimers and which do not react with conventional anti-Ig antibodies or with antibodies directed against Lewis MHC antigens (which presumably include activity against both Ag-B and I-region-associated (Ia) antigens). Moreover, they reported that these in-

herited idiotypes are genetically linked to a Lewis Ig H chain allotype, indicating that the idiotypic-bearing receptors on T cells are coded for in the H-chain variable region (VH) gene pool. Since the isolated T cell receptors seem not to be tightly associated with other polypeptides of different molecular weight, the simplest view is that single or dimeric Ig H chains, having a previously undescribed constant region, serve as receptors for antigen on T lymphocytes.

The findings reported by K. Eichmann (German Cancer Research Centre, Heidelberg) and K. Rajewsky (University of Cologne) and their colleagues were consistent with this view. They had previously used anti-idiotypic antibodies raised in guinea pigs to show that both T helper cells and B cells in A strain mice use the same inherited VH genes for responding to Group A streptococcal carbohydrate⁵. They have now found that only those anti-idiotypic antisera that recognise idiotypes genetically linked to H chain allotypes (and which are therefore related to the H chain alone) are able to stimulate (prime) both T and B cells *in vivo*, whereas those that react with idiotypes that require light (L) chains for their expression can only stimulate B cells. In addition, using hapten-coupled nylon mesh to purify rabbit lymphocyte receptors, they reported the presence of VH allotype determinants on putative T cell receptors which bind antigen but fail to react with antibodies against constant region antigenic determinants of Ig L or H chains.

The other side of the T cell recognition puzzle concerns the target antigens that T cells see. There is increasing evidence that T cells only respond maximally to conventional antigens when they see them in association with MHC antigens. Thus, in order for cytotoxic T cells to respond optimally to viral⁶, haptenic⁷ or minor histocompatibility^{8,9} antigens on mouse target cell surfaces, they must see them in association with H-2D or H-2K gene products; T cells that proliferate in response to antigen-coated "macrophages" *in vitro*, recognise antigen in association with the Ia antigens of the "macrophage" (ref. 10 and W. Paul, National Institutes of Health); helper T cells seem to see antigen in association with Ia antigens (C. Pierce, Harvard Medical School; M. Feldmann and

P. Erb, University College London; H. Von Boehmer (Basel Institute for Immunology) and J. Sprent (University College London)), and T cells responsible for delayed hypersensitivity see some antigens (foreign gamma globulin, for example) in association with Ia antigens, and others (such as dinitrofluorobenzene) in association with H-2D or H-2K gene products (J. Miller, Walter and Eliza Hall Institute, Melbourne).

In most of these situations, the associative recognition of MHC antigens can involve syngeneic or allogeneic MHC determinants; however, the MHC context (syngeneic or allogeneic) in which antigen is first seen by T cells in a primary response is "remembered" by the primed T cells and must be the same MHC context if these cells are to give a secondary response to the antigen. This has been shown for cytotoxic T cells (Zinkernagel and Doherty; Von Boehmer and Sprent), helper T cells (Von Boehmer and Sprent; Pierce; D. Katz, Harvard Medical School) and T cells mediating delayed hypersensitivity (Miller), and explains most experimental results which had previously been considered examples of "syngeneic preference", where it was thought that T cells and macrophages¹⁰, or T cells and B cells¹¹ had to be syngeneic for I-region determinants in order to collaborate. The meshing of the findings in all of these T cell systems is both comforting and exciting. Together, the observations underline the central importance of the MHC in T cell recognition, which must be one of the most important advances in immunology in recent years.

What does this mean in molecular terms? If the same inherited VH genes are used by helper T cells and B cells in A strain mice to recognise Group A streptococcal carbohydrate⁵, and the helper T cells (but not B cells) recognise this antigen in association with Ia determinants, then it seems highly probable that the T cells use a different gene product for recognising the Ia antigens. Since, so far, allelic exclusion has only been shown for B cells it is conceivable that two different VH gene products are involved, one maternal and one paternal. It seems more likely, however, that T cells make two genetically independent receptor molecules, just as B cells make genetically unlinked L and H chains; unlike the B cell receptors, however, the two T cell receptors may not be physically associated on the cell surface. More evidence for two independently coded T cell receptors is provided by Heber-Katz and Wilson¹² who found that T cells positively selected for reactivity against MHC antigens are as effective as unselected T cells in helping B cells respond to sheep erythrocytes. The

pressing need now is to identify the putative "second" T cell receptor; less pressing is the need to establish whether the "antigenic" and MHC determinants have to be physically associated, either on the target cell or "macrophage" surface, or as a shed complex¹³. In any event, the notion that T cells look primarily at a single compound determinant, in the form of antigen-modified MHC glycoproteins ("altered-self")¹⁴ seems improbable.

If there are two classes of antigen-specific receptors on T cells, the undefined "second" receptor presumably recognises mainly self and foreign MHC antigens. Yet, Binz and Wigzell report¹⁵ that 3–5% of Lewis T cells label with their anti-idiotypic antibodies, which are thought to react primarily with those VH gene products that recognise BN MHC determinants. Therefore, it seems that both the VH and the putative "second" T cell receptor gene pools may be heavily committed to recognising MHC antigens and that Jerne¹⁶ may have been right—yet again.

With the demonstration that Ia antigens are important in associative recognition by T cells, the list of I-region functions continues to grow. Various Ia antigens have now been identified on the surfaces of B cells and "macrophages" (see ref. 17), suppressor (D. Murphy and L. Herzenberg, Stanford; M. Feldmann, University College London) and helper (Murphy and Herzenberg) T cells, on enhancing and suppressing factors probably produced by macrophages (Feldmann and Erb) and T cells (T. Tada, Chiba University, Japan; and ref. 18), and on acceptor sites for such factors on T (Tada; Feldmann and Erb) and probably B lymphocytes¹⁸. The simplest view is that most, if not all I-region loci are involved with T cell responses or T cell regulation of B cell function, and that the I-region Immune response (Ir) genes, which regulate T-cell dependent immune responses to a variety of specific antigens, exist at many, if not all, of these different loci, and do not primarily code for T cell receptors. That immunity to a single antigen can be controlled by more than one Ir gene has already been established (B. Benacerraf *et al.*, Harvard Medical School; and refs 18–21) as has the fact that these genes can be expressed in macrophages (or in cells that copurify with macrophages)¹⁹.

Until now, the biology of T lymphocytes has lagged well behind that of B cells—but the pendulum has reversed its swing. Unravelling the functions, biochemistry and genetics of the products of the MHC, now recognised to be central to T cell function, is providing most of the action in immunology these days and the Cold Spring

Harbor Symposium was a celebration for those involved.

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Finding a niche

from Peter D. Moore

THERE are few terms in ecological jargon which are used more frequently than the 'ecological niche' and there are few which have been used so loosely. This is particularly true in plant ecology where there have been few attempts to construct working definitions. The use made of the term by Elton (*Animal Ecology*, Sidgwick and Jackson, London; 1927) was essentially applicable to animals and referred to the role of a species in a community as far as its feeding and competitive interactions are concerned. In autotrophic plants the variety of functional roles available is obviously severely limited, so the Eltonian concept of niche has not proved particularly valuable.

Hutchinson (*The Ecological Theater and the Evolutionary Play*, Yale University Press, New Haven; 1965) has provided a concept of 'niche' which is of much greater value to plant scientists. If one considers all the possible environmental factors which influence a species, and the organism's response to these factors, then these can be regarded as axes in multi-dimensional space. Each species thus occupies a definable volume of 'hyperspace', which represents the set of conditions under which the species can survive. This potential or 'fundamental' niche, may not be identical to its 'realised' niche, for competitive interactions between species whose fundamental niches overlap may result in their restriction within their potential volume. Wuen-scher (in *Handbook of Vegetation*

Science No. 6, edit. by Strain, B. R. and Billings, W. D., 39, Springer Verlag, Berlin, 1974) has discussed the Hutchinsonian concept of niche as it applies to plants and has also stressed how the concept goes beyond that of simple habitat. Any point within niche hyperspace can be described by the co-ordinates of all the environmental variables (habitat factors) and the response of the organism to these factors. The response element carries the niche concept beyond that of habitat alone.

In a community it is to be expected that the component species will occupy adjacent hyperspace volumes, since their basic habitat requirements will be similar. In this case, the introduction of a new species can only be successful if other species are permanently displaced from at least part of their former niches. Total displacement will, of course, result in extinctions. An example of this situation was described in some detail, by Zaret and Paine (*Science*, **182**, 449; 1973) following the introduction of an exotic cichlid fish, *Cichla ocellaris*, into Gatun Lake in the Panama Canal Zone. This predatory species caused considerable simplification in community structure by supplanting several native species. Examples from the plant kingdom are not as well documented, but they certainly exist. The abundance of the sycamore (*Acer pseudoplatanus*), introduced into British woodlands probably in the early sixteenth century, has almost certainly been achieved only at the expense of native species, but it would be difficult to prove this.

There are situations in which an introduced species appears to have no deleterious influence upon the native components of a community. If such a situation does occur, it implies that the niche of the invading species has no volume of overlap with those of the natives. It has recently been claimed by Hudson (*Brit. Birds*, **69**, 132; 1976) that the North American ruddy duck (*Oxyura jamaicensis*) which has become feral in Britain over the past 30 years, has occupied a 'vacant niche' in that its ecological requirements overlap little with such native diving ducks as the pochard (*Aythya ferina*) and tufted duck (*A. fuligula*). In Hutchinsonian terms, the niche can only be defined in terms of environment and species response, so strictly one should not speak of 'vacant niches', however the idea of an untapped environmental resource is often referred to in this way. The former vacancy in hyperspace can only be recognised in retrospect.

Danin (*Oecologia*, **22**, 251; 1976) has recently conducted a survey of annual plant species under desert conditions in the Dead Sea Valley of Israel. He showed that diversity falls with increas-

MAN'S first picture of the surface of Venus caused great interest and some surprise as it revealed that Venus, like Mercury, Moon and Mars, had a cratered surface. The picture was obtained using simultaneous Doppler and time delay processing of 12.6 cm wavelength radar echoes reflected from the surface (Rumsey *et al.*, *Icarus*, **23**, 1; 1974). Looking at a 1,500 km diameter circular area at the centre of the planetary disk on June 20, 1972, they found it to be nearly flat but cratered. The biggest crater was 160 km in diameter, the floor appearing to be on the same level as the surrounding terrain but with the rim 500 m above the floor. By contrast a lunar crater of this size would be about seven times deeper.

Venus and Earth have fairly similar masses and both seem to have large raised regions on their surfaces. The "continents" on Venus do not appear to be isostatically compensated so that active internal convection may be taking place coupled with a degree of surface volcanism. Craters will be formed both by volcanic activity and by meteoritic activity; however, the information needed to determine which phenomenon is more likely is still lacking.

Michael Tauber and Donn Kirk (NASA-Ames Research Center) have now attempted to determine the sizes of the stone and iron meteoroids which can penetrate the atmosphere of Venus and cause hypervelocity impact craters on the planet's surface (*Icarus*, **28**, 351; 1976). Obviously the surface pressure of 90 atmospheres and the atmospheric density of 64 kg m^{-3} present a major obstacle to the passage of meteoroids. Ninety percent of the meteoroids will have atmospheric entry velocities ranging from 10 to 40 km s^{-1} and if all of the kinetic energy lost by atmospheric

drag was available to heat and vapourise the meteoroid no meteoroids less than 10^{11} kg in mass would survive passage through the atmosphere. Actually only a small fraction of this energy goes to heat the body, the great majority's being lost as it heats the atmospheric gases. The complex interactions between the body and the high temperature-high pressure shock layer surrounding it make the mass

Venusian craters

from David W. Hughes

loss calculations very difficult. Tauber and Kirk find that bodies over 10^8 kg only lose a few percent of their mass by vapourisation, mainly because entry takes place so quickly. The velocity of the body can decrease drastically though.

A hypervelocity impact crater is produced when the shock pressure at the impact point exceeds the compressive dynamic strength of the target material. Venera 8 measurements suggest that Venus has a granite-like surface material and the authors calculate that basalt and iron projectiles with velocities greater than 1.1 km s^{-1} and 0.7 km s^{-1} will produce hypervelocity impacts and craters. For basalt and iron particles they find that it is only those larger than 100 m and 40 m that have final velocities above these limits. One complication is the possible mechanical break-up of the body before impact. This is caused by a combination of thermal stress from frictional heating and also air pressure acting on the high speed body as it is retarded in the atmosphere. Frictional heating is confined to a thin shell and probably does not affect large meteoroids. The atmospheric forces on the other hand become very large and may exceed the compressive strength. It seems

that for large bodies this only happens for a second or so before impact so there is not enough time for the cracks to spread throughout the body or for fragments to separate significantly. So on Venus this effect does not change the size of the crater produced. The authors calculate that a stone meteoroid 1 km across, entering the Venusian atmosphere at 40 km s^{-1} will produce a crater 33 km in diameter. This diameter is proportional to the sixth power of the particle's density and its kinetic energy raised to the power 0.28. They also find that the smallest hypervelocity impact crater on the surface of Venus is about 150 to 300 m across. This contrasts dramatically with the Moon which has no atmosphere to retard or fragment the meteoroids and is pockmarked with craters of all sizes greater than a fraction of a micrometre.

Venera 8 measurements (*Icarus*, **20**, 407; 1973) indicate very low surface winds on Venus so the crater structure may be preserved for long periods of time. The radar picture taken by Rumsey *et al.* had a resolution of about 12 km, so it cannot be used to test Tauber and Kirk's theory. Radar mapping from a planetary orbiter, or radar using the Arecibo radio telescope could provide the resolution. In fact photography from a probe descending through the Venusian atmosphere is possible as the daylight illumination level at the surface is thought to be about 2% of that on Earth. The discovery of primary impact craters smaller than the 150-300 m calculated above would indicate that the impacts occurred when the atmosphere of Venus was less dense than it is now. So crater size distribution thus provides a straightforward indication of atmospheric evolution.

ing soil salinity until eventually, in highly saline conditions, only one species, *Mesembryanthemum nodiflorum*, survives. Danin describes this gradient as one in which a physiological stress reduces the number of niches until, under extreme stress, a one niche habitat results. This situation does not preclude competitive interplay, but demonstrates the superior growth potential of the surviving species under the stress conditions. In the Hutchinsonian sense it can thus be termed a one niche habitat. What one must not discount, however, is the possibility that there could be unrecognised opportunities even here for species which have either not yet invaded, or may not even yet have evolved in the area. Could one term such an availability of

opportunity, such untapped resource potential — such unoccupied hyperspace, a vacant niche? □

Nuclear polysomes

from J. R. Tata

CELL biologists often emphasise the intracellular segregation of transcription and translation between the nucleus and cytoplasm as a major distinguishing feature between eukaryotes and prokaryotes. Every so often, however, fresh controversy is generated by reports that challenge the validity of this intracellular compartmentation of macromolecular synthesis by demon-

strating that nuclei can also synthesise proteins (for a review, see Kuehl, in *The Cell Nucleus*, edit. by Busch, H., **3**, 345; Academic Press, New York, 1974). Many such claims have had a short life since these are based on indirect evidence, or fail to identify a special class of proteins synthesised within the nucleus. Perhaps the most important reason for the controversy over whether or not the nucleus possesses a protein synthetic apparatus may reside in the ease with which isolated nuclei are contaminated with cytoplasmic polyribosomes (Penman, *J. molec. Biol.*, **17**, 117; 1966), particularly those associated with the perinuclear endoplasmic reticulum (Lewis and Tata, *J. Cell Sci.*, **13**, 447; 1973).

Jo Alene Goidl and coworkers at the

Roche Institute in New Jersey have now managed to demonstrate polyribosome-like structures from isolated HeLa cell nuclei meticulously freed of contaminating cytoplasmic polysomes by detergent treatment (Goidl *et al.*, *J. biol. Chem.*, **250**, 9198; 1975). Exposure of these nuclei to polyanions (heparin, RNA and particularly poly (U)), which are known to disrupt nuclear structure (Coffey *et al.*, *Adv. Enzyme Regnt.*, **12**, 219; 1974), caused the release of particles of 200–300 Å in diameter. These unique particles were shown, in a heterologous translation system derived from Ehrlich ascites cells, to comprise mRNA or precursors of mRNA associated with ribosomes and the complex itself was shown to be able to carry out translation. Goidl and her colleagues also found that poly (U) used for the disruption of nuclei was not associated with these nuclear polysomes; yet they were capable of polypeptide chain elongation but not initiation, as judged by effects of inhibitors of cell-free protein synthesis. These findings may have some relevance to those of Bachellerie *et al.* (*Eur. J. Biochem.*, **58**, 327; 1975), who characterised transcription complexes of ribosomes and particles containing HnRNA still bound to chromatin derived from nuclei of ascites hepatoma and Chinese hamster ovary cells. The question of whether such complexes synthesise proteins within the nucleus or whether they are “packages” for transport of mRNA into the cytoplasm, as Harris (*Nucleus and Cytoplasm*, Clarendon Press, Oxford; 1974) suggested, is still open. Be that as it may, in view of the past controversy about protein synthesis by isolated nuclei, Goidl *et al.*, quite prudently, still do not state categorically that their polyribosomal material is exclusively nuclear but imply that if these complexes are of cytoplasmic origin, then their release from the nucleus somehow requires disruption of chromatin.

In a wider context, two questions concerning the capability of protein synthesis by nuclei have now to be considered. First, if they do have this function, do they make special classes of proteins? There are periodic but often unconfirmed reports of such special proteins exclusively made in the nucleus, but it is almost certain that the major nuclear proteins (histones, acidic chromosomal proteins, DNA and RNA polymerases) are synthesised in the cytoplasm. Second, what is the likely magnitude on a cellular basis, of protein synthetic activity in the nucleus? By isolating detergent-washed nuclei from sea urchin blastula cells, labelled *in vivo* with ³H-leucine, Allen and Wilt (*Expl Cell Res.*, **97**, 151;

1975) concluded that no more than 0.2% of the total cellular nascent polypeptides could be recovered with the nuclei. Perhaps this figure may be higher for nuclei or other cells, such as thymus or nucleated erythrocytes, but it is still likely to be quite low. Whatever the extent of protein synthetic activity of the nucleus, Goidl and her colleagues have certainly reopened, and with convincing evidence, an old controversy. Past critics will certainly find new reasons to disbelieve translation of any RNA within the nucleus. One has only to go back fifteen years to recall the controversy raging around protein and nucleic acid synthesis in mitochondria to draw a useful lesson and keep an open mind on this unsolved problem. □

Estimating maximum quake magnitudes

from Peter J. Smith

ACCORDING to a well known empirical formula, the number (N) of earthquakes per unit time exceeding any magnitude (M) is given by $\log N = a + bM$. Both a and b are usually described as positive constants, although they are not universally constant; a varies greatly from region to region and b , though generally more restricted, varies with region, focal depth range and in some areas even with magnitude. Irrespective of these variations, however, implicit in the general relationship is the existence of an upper limit on earthquake magnitude. If a and b are taken to be the averages for worldwide earthquakes, this limit turns out to be about 9.0.

It would appear from the work of Perkins (*Earthquake Inf. Bull.*, pp 18–23, July 1972), and others that this maximum magnitude may be established on the basis of worldwide earthquake statistics covering the period of only the past 50–70 yr, during which time it may safely be presumed that at least one event of roughly maximum magnitude has actually occurred (for example, the magnitude 8.9 earthquakes of Colombia in 1906 and Japan in 1933). Unfortunately, the establishment of a global upper magnitude is largely of academic interest. A more common practical problem (for example, in the field of earthquake engineering) is the assessment of the likely maximum magnitude of earthquakes within a restricted local region where the seismic statistics are but a depleted subset of the global total.

In local areas where earthquakes are clearly associated with faults, a very rough estimate of likely maximum magnitude along a given fault may be

made from curves of fault length against magnitude based on recent seismic experience. Unfortunately, however, the correlation between earthquake magnitude and surface rupture is poor and varies with fault type. In regions where the faults are less clear, the prediction of the upper limit is even more uncertain; it can only be assumed that the earthquake of highest magnitude ever recorded in the area could be repeated anywhere within it. The drawback of such a method is obvious. An earthquake of the highest possible magnitude for any given zone may not have been recorded in the zone in historic time. Moreover, the period covered by local seismic statistics may be quite insufficient to define an expected maximum magnitude on the basis of the formula involving a and b .

But how long a sampling period would be adequate? According to S. W. Smith (*Geophys. Res. Lett.*, **3**, 351; 1976), if the Earth's major seismic zones with a total length of about 80,000 km produce several maximum magnitude earthquakes in 50 yr, then 20,000 yr of seismic monitoring would be needed to give comparable statistics on a local scale of, say, 100 km. Needless to say, such long term monitoring is out of the question. On the other hand, it is quite feasible in principle to obtain fault displacement over a comparable period from the geological record. What is required, then, is some way in which, in the absence of an adequate seismic record, the geological record may be made to yield an estimate of maximum seismic magnitude.

The essential link between geological data and earthquake statistics, Smith proposes, is seismic moment. As defined in the dislocation theory of faulting, the seismic moment of an event is the product of rigidity, fault area and the average slip over the fault during the event. This relationship was exploited some years ago by Brune (*J. geophys. Res.*, **73**, 777; 1968) who used it to estimate the total slip along major fault zones by summing the seismic moments of recorded earthquakes. Smith now reverses the process by using the geological record of total fault slip to determine the total historic seismic moment on a fault. This total moment is then fed into an expression for maximum magnitude obtained by integrating the moments of all earthquakes along the fault up to the maximum magnitude.

Inevitably, such a process involves assumptions. One is the taking of an empirical relationship of the form $\log Y = c + dM$ between moment (Y) and magnitude (M). Another is the validity of the original N – M relationship. It must also be assumed that the

seismicity is a stationary process on a time scale of thousands of years and that during those years the maximum earthquake has occurred at least once. Finally, implicit in the method is the assumption that the observed geological displacement along the fault is due to earthquakes rather than creep, for any significant component of creep would lead to an overestimation of the maximum expected magnitude.

The need for these assumptions and natural deficiencies in the basic geological data combine to ensure that the method is less than perfect. On the other hand, as Smith points out, it does have the merit over existing techniques of making greater use of geological information. In any event, application of the method predicts maximum magnitudes of 8.2–8.4 for the first order branches (for example, the Calaveras fault) of the San Andreas fault system, 6.3–6.5 for lesser branches (for example, San Simeon) and 6.3 for still lesser branches (for example, West Huasna). Whatever the validity of these figures (compare magnitude 8.3 for the 1906 San Francisco shock) they are certainly more precisely defined than those obtained from fault length-magnitude curves. For a 200 km fault, for example, the conventional technique would only give an upper limit in the looser range 7.3–8.5. □

Poly(ADP-ribose)

from Mark Smulson and Sydney Shall

The fourth International Workshop on poly(ADP-ribose) was held in Hamburg on August 2–4, 1976, was sponsored by the Deutsche Forschungsgemeinschaft and organised by Professor Helmuth Hiltz.

THE enzyme poly(ADP-ribose) polymerase requires DNA and catalyses the successive transfer of ADP-ribose units from NAD to histones and other proteins associated with chromatin, the net result being negatively charged, short polymers covalently attached to nuclear proteins.

The workshop concentrated on three main themes; possible biological roles for this modification, definition of the nature and chemistry of the protein acceptors of the polymer, and the purification and characterisation of the enzyme itself. The most exciting advance in the area was experiments suggesting that poly(ADP-ribose) cross-links chromosomal proteins. P. R. Stone and W. R. Kidwell (National Cancer Institute, Bethesda) reported that after incubation of HeLa cell nuclei with NAD, a dimer of histone H1 can be isolated containing one link-

ing chain of 15 ADP-ribose residues. Further evidence for this structure was provided in the previous week in a lecture at the International Congress of Biochemistry in Hamburg by G. Dixon (Calgary Medical School, Canada): he suggested either an inter- or intramolecular covalent linkage by way of poly(ADP-ribose) between glutamic acid residues near the amino terminal and carboxyl terminal ends of trout sperm histone H1. Since the concentration of poly(ADP-ribose) in intact HeLa cells reaches a maximum at the S-G2 phase boundary, Kidwell suggested that the crosslinking property of poly(ADP-ribose) for histone H1 (and perhaps non-histone protein acceptors as well) might function to link widely spaced H1 molecules along internucleosome regions and hence condense chromatin. This model was supported by the finding (M. Smulson, Georgetown University, Washington) that enzyme activity can be detected in internucleosome regions of chromatin and not on isolated nucleosomes.

Extensive purification of the enzyme has been accomplished independently in at least four laboratories. Okayama, Ueda and Hayaishi (Kyoto) have found during their 7,000-fold purification that an endogenous acceptor for ADP-ribose copurifies with the enzyme; and it may not be a simple protein. Histones decrease the K_m for NAD in their preparation. Confirmatory results were reported by K. Yoshihara (Nara Medical School, Japan). Both S. Shall (University of Sussex) and P. Mandel (Centre de Neurochimie du CNRS, Strasbourg) suggest the loss of an inhibitor of the enzyme during their purifications. In addition, Shall and coworkers have estimated the K_i for a number of poly(ADP-ribose) polymerase inhibitors which turn out to be also cyclic AMP phosphodiesterase inhibitors, with higher affinity for the poly(ADP-ribose) polymerase, suggesting that some biological effects attributed to cyclic AMP from inhibitor studies, might really be due to poly(ADP-ribose). Certain NAD analogues were reported to be incorporated into poly(ADP-ribose) chains (R. Suha-dolnik, Temple University).

Various new data on chromosomal protein acceptors for poly(ADP-ribose) were presented. M. G. Ord and L. A. Stocken (University of Oxford) find that histone H3 as well as H1 has ADP-ribose. Hiltz and coworkers (Hamburg, Germany) have found heterogeneity both in the acceptors among non-histone proteins and in the sensitivity of the covalent linkage to cleavage. The proportion of monomer and polymer varies with changes in cellular proliferation rates. M. Miwa and T. Sugimura (National Cancer Centre, Tokyo) have been able to detect poly-

mer chains with equal (ADP-ribose) units but with different phosphate termini by acrylamide gel electrophoresis.

One frustration has been the heterogeneity in the "tentatively" reported covalent linkage of the first ADP-ribose to histones. The proposed linkages include attachments from C-1 ribose to glutamyl carboxyl and threonyl hydroxyl by way of glycosidic bonds, to seryl phosphate by way of an ester bond or N-glycoside to arginine, and a possible Schiff base linkage. Much work clearly needs to be done in this area, but the heterogeneity of acceptors between histones and non-histone proteins suggests that various covalent attachment sites will ultimately be established.

The development of new ultrasensitive assays, including fluorometric determination (Mandel) and radioimmunoassays (Miwa and Sugimura; Bredehorst and Hiltz; Kidwell) for quantitation of poly(ADP-ribose) in cellular extracts, have produced substantial, but still far from definitive progress towards assigning a biological function to this fascinating chromosomal protein modification system. Reports at the workshop suggest that ADP-ribosylation, like other nuclear protein modifications such as phosphorylation, probably has multiple functions all revolving around specific large or small structural perturbations of chromatin. Increases in enzyme activity are noted in SV40 transformation of cells (M. Miwa and T. Sugimura) for example; in induction of globin mRNA in Friend cells (E. Rastl, Ernst-Boehringer-Institut, Vienna) and an interesting correlation was described by A. Caplan (Case Western Reserve University, Cleveland) between intracellular NAD levels, poly(ADP-ribose) polymerase activity and the differentiation of mesenchymal cells either to muscle or cartilage.

Experimental approaches which make use of the effect of cytotoxic DNA-alkylating agents on poly(ADP-ribose) polymerase activity and *vice versa* also indicate that poly(ADP-ribose)-induced changes in chromatin structure may be important in repair of DNA damage. Fragmented sites seem to be a signal for increased *in vivo* activity of poly(ADP-ribose) polymerase (groups at Georgetown and Sussex).

On the basis of data showing accumulation of poly(ADP-ribose) in mid-S phase and again in the G2 phase of the cell cycle (Kidwell) there was also speculation that chromatin repair and housekeeping must occur after the first peak of S phase, perhaps by way of polyADP-ribosylation relaxation of chromatin, and again in early G2 phase for cells to complete a normal cycle.

review article

Background of modern comet theory

Fred L. Whipple*

The modern understanding of comets dates from the publication in the early 1950s of three crucial ideas: that of a comet cloud around the Sun, that of the dirty-ice comet nucleus, and that of the "solar wind" to explain the comet tail. Starting from those three ideas, the observations of the past 25 years have led to the explanation of all the mysterious and sometimes spectacular phenomena associated with comets.

ANCIENT records indicate that extremely bright comets terrified almost everybody. Their erratic, unpredictable and mysterious activities seem to have touched off a basic human fear of the unknown, not just a fear of dangerous atmospheric objects which comets were assumed to be. This primitive fear, no doubt exploited by man's spiritual advisors throughout history, was still being exploited in this century. Entrepreneurs, for example, sold "comet pills" as preventative medicine against the noxious vapours from Halley's comet during its apparition in 1910. Chinese astronomers, incidentally, recorded Halley's great comet in 239 BC and probably as early as 467 BC¹.

Tycho Brahe of Denmark was the first to undermine the basis for this fear. He removed comets from the upper atmosphere and placed them in the sphere of modern scientific research by proving that the bright comet of 1577 moved in interplanetary space at a greater distance from Earth than the Moon. Sir Isaac Newton then applied gravitational theory to comets and calculated the first periodic orbit for a comet—that of 1680. Applying Newton's methods, Edmund Halley first predicted correctly the return of a comet, alas posthumously, when he identified the bright comet of 1682 as the return of comets seen in 1607 and 1531. His words: "therefore, if it should return according to our predictions about the year 1758 impartial posterity will not refuse to acknowledge that this was discovered by an Englishman." Hereby acknowledged!

Since about 1700, the discovery, the meticulous observing and the calculating of orbits for comets has been a major astronomical enterprise. Marsden's² *Catalogue of Cometary Orbits* lists 21 orbits in the seventeenth century; 63 in the eighteenth; 308 in the nineteenth; and 513 in the twentieth (until 1975). In all, the catalogue includes 964 apparitions of 625 individual comets of which 102 have periods less than ~150 yr. One must admire and be grateful for the patience, persistence and careful workmanship of the cometary astronomers who have given us so much invaluable basic information. But a knowledge of the precise orbits and appearance of many comets was only a necessary prelude to understanding their true nature. The phenomena of comets are produced by physical and chemical processes, which had to be ferreted out step by step. Some of these major steps are described in this paper.

Kepler, although he did not understand the orbital motion of comets, sought to explain earlier observations that comet tails point away from the Sun "by the supposition that a

tail is formed by rays of the Sun which penetrate the body of the comet and carry away with them some portion of its substance."³ He thereby anticipated the phenomenon of solar radiation pressure as a major factor in the formation of comet tails, although the phenomenon itself was not demonstrated theoretically until much later by Clark Maxwell, and in the laboratory by Lebedev in 1901. Bessel, in 1836, initiated the mathematical analysis of a solar repulsive force on particles ejected from comets and forced into their tails, although he looked on the force as electrical or magnetic. Bredikhin, in 1903, further developed and applied the theory to account for the observed highly curved dust tails. Theoreticians, however, remained frustrated in their attempts to explain motions in the great straight tails by means of solar radiation pressure alone. Accelerations exceeding a hundred times solar gravity, even on light ionised atoms or molecules, require a force that is much better coupled than solar radiation.

Schiaparelli, in 1866, made a major step in understanding comets when he identified the orbit of the Perseid meteor stream of August with that of Tuttle's comet of 1862, thus proving that comets lose solid bodies and therefore must contain them intrinsically. Two years earlier Donati made the first spectroscopic observations, a start towards the later identification of the bands and lines of radicals, atoms and ions and of the continuous spectrum reflected by dust. By 1911 Schwarzschild and Kron had identified the fundamental fluorescent process by which the band spectra are produced. Solar radiation is absorbed in ground energy levels of atoms or molecules, producing excitation. Fluorescence or reradiation then follows by cascading from high energy levels through various lower levels. Because of low space densities and dilute solar radiation the higher energy levels are erratically filled, depending on the intensity of the solar radiation at the specific wavelengths of transitions from the ground levels. Thus the relative intensities of lines in the band spectra are distorted from their laboratory counterparts. Swings⁴ demonstrated in 1941 that the variable Doppler effect of a comet's velocity with respect to the Sun changes the critical wavelengths absorbed from the solar spectrum by cometary particles. Irregularities in the spectra then produce variations in the cometary spectra so that the distorted bands change their appearance with time.

Thus in the 1940s it was recognised that bright comets such as Halley's near perihelion (0.59 AU: 1 AU = the Earth-Sun distance) lose the order of $10,000 \text{ t d}^{-1}$ of C_2 alone⁵. The total loss rate had to be increased by the other observed contributing radicals, such as CN, CH, CO, OH and NH, amounting altogether to a moderate fraction of a

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ton per second! Estimates of the meteoroidal losses as evidenced by meteor streams were smaller but of comparable magnitude, while the spectra of meteors showed the generally abundant heavier elements Fe, Ca, Mg, Mn, Cr, Si, Ni, Al and Na. Meteorites, however, did not seem to be of cometary origin as no falls had been observed from cometary meteor streams.

Erratic variations in cometary brightnesses had always been known, and careful observations had shown that the relationship between intrinsic brightness and solar distance (r) varied greatly from comet to comet, the average relationship being $\sim r^{-3}$ to $\sim r^{-4}$. A few comets had disappeared while others had split into two or more distinct components accompanied by a "burst" of luminosity, usually to disappear later. For the Sun-grazing family, however, a major component usually persisted to a considerable distance from the Sun. Encke's comet had made more than fifty revolutions with a supposedly (but erroneously high) diminution of some ten times in brightness.

Most of these facts and phenomena fit qualitatively with Proctor's late nineteenth century concept of the comet nucleus as a sand bank or gravel bank. He pictured the fundamental particles as solid meteoroids from which absorbed gases are released by solar heating, the rate of gas desorption increasing rapidly with decreasing solar distance. The observed phenomena are evanescent, resulting from solar radiation acting in various ways on the gas and dust.

Attempts to define such a gravel bank quantitatively, however, led generally to increasing difficulties or contradictions. The nature of the dilemma can be seen qualitatively. One extreme is the nearly transparent gravel bank with negligible gravitational cohesion. The particles are all exposed to sunlight and must lose most of their gases on the first heating. Unless metres in dimension, they must be vaporised and entirely lost for the several Sun-grazing comets. Because each particle moves in its own orbit about the Sun, particles of different sizes will be forced into orbits of different periods by the Poynting-Robertson effect of solar radiation. Tus comets, especially periodic comets, should be elongated along their orbits if their nuclei are transparent gravel banks.

At the other extreme, the gravel-bank model is opaque near the centre and massive enough to hold particles gravitationally against solar tide-raising disruption. Because of orbital motions the particles will collide frequently at very low velocities and there is no reason to believe that the collisions will all be elastic. Thus a compact core will develop and become essentially asteroidal in character.

Intermediate gravel-bank models have been designed to meet some of the observational requirements only to stumble in explaining others. A possible hierarchy of such models to account for various types of comets is still no solution because even the fit of a special model to a specific comet is usually unsatisfactory.

Furthermore, a sinister persistent threat to the various gravel-bank models lay in Encke's (1823) discovery that the comet of shortest period was measurably decreasing in period, some 2 h in 3.3 yr. During the following century, this comet, which had been named after Encke, slowly reduced its rate of period decrease, that is, its deviation from gravitational motion. The popular postulate of a resisting medium between the planets, tempting as it may have been in the eighteenth century, became progressively less tenable in the nineteenth because no other supporting evidence could be found and because improved knowledge of interplanetary space impressed progressively lower limits on its possible density. The death knell for the idea sounded when two other periodic comets showed positive accelerations in their non-gravitational motions. A resisting medium can only reduce the dimensions of an orbit and thus the period. Increasing periods negate the possibility of a resisting medium. Conclusion: some comets must engender intrinsic

forces to change their periods, an idea suggested by Bessel⁶ as early as 1836 and by Dubiago⁷ in 1948, but without realistic physical mechanisms.

In spite of increasing detailed knowledge about comets and the abject failure of the gravel-bank model to fit the data quantitatively, the quantum jump in cometary theory was delayed until the middle of this century. Three explicit new ideas then revolutionised our concepts of comets. The application and development of these ideas led to remarkable advances. These new theories are presented here in the order of their publication and are identified in terms of the cometary phenomena involved.

Oort's great comet cloud

In 1950 Oort⁸ developed the theory of a great comet reservoir, or cloud about the Sun, providing a source of "new" comets to replenish the observable supply. Oort demonstrated that passing stars can perturb comets from huge orbits, extending out perhaps to 40–50,000 AU, into orbits with perihelion passages among the planets. Perturbations by the planets, particularly Jupiter, then eliminate more than half of such comets in successive passages, but disturb some into the short period orbits associated mostly with Jupiter. Oort thus defined specifically "The Home of the Comet"—a vague concept predating him by more than half a century. Although Opik⁹ had long since shown that such a solar attached cloud is quasi-stable with respect to stellar perturbations, he had not specified the retrieval process that brings "new" comets on nearly parabolic orbits into the inner Solar System for the first time.

The dirty-ice comet nucleus

Whipple's¹⁰ theory that the nucleus of a comet is a compact mass of ices with intermingling earthy materials formed a basis for understanding many aspects of comets, including: (1) comet activity by way of the sublimation of ices by solar radiation, (2) loss of large masses of gas and solids from long-lived comets, (3) observed deviations of comets from Newtonian motions, (4) the origin of comets by the aggregation of cosmically-abundant low temperature condensates from typical interstellar or stellar gases.

The solar wind and ion tails

Biermann's¹¹ concept—that solar corpuscular radiation, now generally known as the solar wind, could produce the structural forms and high accelerations observed in the ion tails of comets—provided the key that opened the door to understanding the one most conspicuous phenomenon of comets. These long, nearly straight tails sometimes extend for more than 10^8 km and show accelerations up to 100 times or more of solar gravity. As we have noted, the explanation—solar radiation—that previously had served semi-quantitatively to account for dust tails, curving strongly backwards in a comet's orbital plane, proved quite inadequate when applied to ion tails.

These three new concepts were accepted relatively soon by most of the astronomical community because they promised to fill conspicuous lacunae in the theory of comets. All the major questions regarding the nature and phenomenology of comets could at last be approached by observers, theoreticians and celestial mechanicians on the basis of reasonable working hypotheses, to be subjected to the scientific rigour of checking and improvement. The full blossoming of cometary astronomy then emerged. The subsequent outstanding developments of space science, coupled with highly improved optical, infrared and radio sensors, added a wealth of new information that could be quantitatively integrated with older data.

Here I outline the development of my own ideas about the nature of comets. I first note my faith in a basic general principle to be applied in exploring the frontiers of any field in science. One is attempting to find the way through a

swampy morass of mutually conflicting observations, interpretations and theories. The principle: seize on a few "indisputable facts" that appear most relevant; momentarily ignore the remaining data. On these solid "facts" build conceptual structures or working hypotheses that are self-consistent, strongly rooted in the best evidence available. Then attempt to expand each working hypothesis by fitting in the successively weaker material. Rejection of material must be rigorously monitored. Is it irrelevant? Is it erroneous? Is it misinterpreted because of incomplete theory or because of an unnecessary or erroneous assumption, either tacit or explicit? If none of these, another working hypothesis should be investigated.

The general knowledge of comets available to me in the 1940s is summarised above. But I was highly conscious of three other "solid facts" that had been published but may not have been known or considered important by others who were thinking about comets. The first fact was the clear cut evidence I¹² had amassed from photographic meteor studies that Encke's comet had made not just about fifty revolutions but actually thousands of revolutions. The second fact was the evidence from the polarisation of the Zodiacal Light that interplanetary space at the Earth's solar distance contains less than 10^3 electrons cm^{-3} and, therefore, even fewer atomic nuclei¹³. The third fact was Opik's⁹ demonstration that a cometary cloud could be quasi-stable about the Sun to a distance of several times 10^4 AU. The third fact left me with little concern about the replenishment of the shorter lived comets, because Opik's work, it seemed to me, had implied this likelihood.

The critical factor to me was the large quantity of matter that comets lose to space. If any comet can persist for a thousand or more revolutions, coming to 0.3 AU of the Sun at perihelion, it must contain or acquire a substantial quantity of gas and solids (cubic kilometres?). Conceivably a gravel-bank comet might persist if there were a gas source in space to refuel the solids by absorption. A back-of-the-envelope calculation suffices to show, however, that the known gas content of interplanetary space is woefully inadequate. Given a few km^2 effective area of the gravel bank, based on the brightness of comets at great solar distances, and even a $1,000\text{-km s}^{-1}$ radial velocity for the solar-emitted gas, the "refuelling" situation is hopeless.

Other implications of the gravel-bank theory were totally unsatisfactory to me. As mentioned above, a hierarchy of particle sizes would conspicuously spread the gravel-bank along its orbit in a thousand revolutions. Also I could never visualise a satisfactory process to split a gravel bank in the manner required to account for comet splitting, nor, indeed, a mechanism to produce comet bursts.

A dirty-ice model of the nucleus could, however, carry any specified reserve of solids and potential gas. It could lie dormant at great solar distances and become increasingly active as it approached the Sun. It could withstand the temporary heating experienced by the Sun-grazing comets and, indeed, split tidally to give birth to the family. It could account, at least qualitatively, for almost all of the many phenomena of comets. Furthermore, it was reasonable in terms of cometary origin as a low temperature condensate from typical solar or interstellar material. The most abundant compound-forming elements in a "solar mix", H, C, N and O provide the atoms in the radicals producing cometary fluorescence, while the heavier elements would certainly freeze out if the more volatile gases could. In principle, then, the basic difference between the conditions for the origin of the terrestrial planets and the comets become one of temperature.

At this stage of my thinking I felt that the icy conglomerate model was so obvious that it must already have been tacitly accepted by the major students of comets. There seemed to be little justification for publishing the idea unless some quantitative aspects could be developed. In a remark-

able treatise Hirn¹⁴ had essentially demolished the idea of a resisting medium because of its resultant effect on comets' tails, and concluded that cometary gas would be frozen at aphelion to "*forme alors un assemblage de corps solides désagregés*." Pol Swings, indeed, who was impressed by my evidence for the great age of Encke's comet, almost presented the icy model in 1948 (ref. 15). He concluded that the gas of a comet occurs by evaporation, not by desorption, but failed to develop the concept further, being constrained by the classical assumption that a comet nucleus consists of numerous small bodies and that breakage is important to produce activity. In retrospect it seems that this persistent assumption of a multibodied comet nucleus was the major obstacle to an earlier development of the icy concept. I was not so constrained because I realised that solids imbedded in a small icy body of low surface gravity would be blown out aerodynamically by sublimating gases. The novelty of this idea did not occur to me at the time and thus I abandoned the comet problem for a year or two.

I decided to publish the icy comet model while working on a theory (unpublished) of meteor drag in the atmosphere. I was attempting to include a term in the classical drag theory to allow for the jet action of vaporising meteoroidal material. Suddenly I realised that an icy comet nucleus should produce just such a jet action. Furthermore, any delay in the sublimation process for a rotating nucleus would produce a jet force component normal to the solar direction and thus accelerate or decelerate the orbital motion, depending on the sense of rotation. Here was a quantitative mechanism for explaining non-gravitational motions in comets with either positive or negative rates of period change. Rotation might be expected for any solid body in space, but certainly could be produced in an irregularly shaped icy nucleus. The integrated force vector of the jet action need not point precisely at the centre of gravity. Thus the model could explain comet splitting as a spinup phenomenon, as well as a tidal disruption.

A major problem of comets was and, to some extent still remains: the identity of stable "parent" molecules to be dissociated into the observed radicals. Not being a chemist I was content with the previously suggested H_2O , CH_4 , methane- and NH_3 (ammonia) to account for OH, CH and NH, respectively, with CO_2 (carbon dioxide) or CO for CO. The radical CN was more difficult because the obvious C_2N_2 (cyanogen) is so active. I favoured HCN, or C_2H and N_2 requiring some chemical action to produce CN. The chemists were unhappy with these "non-equilibrium" chemical companions but I felt (hoped) the compounds might bed together at very low temperatures. The extremely low temperature ($\lesssim 20\text{ K}$), required to freeze out CH_4 , CO or N_2 worried me more. This problem was resolved by Delsemme and Swings¹⁶ in 1952 who suggested that the highly volatile CH_4 and others might be imbedded in H_2O snow as hydrates or clathrates. Their suggestion fits the observations well and has generally been accepted.

The most likely parent molecule should be H_2O because of its high heat of vaporisation. It should also be the most abundant. The luminosity variation of short period comets clearly indicated its presence. At a solar distance of 2.5–3 AU the vapour pressure of H_2O in sunlight becomes negligible as does the activity of most of these comets.

Calculations¹⁰ based on the assumption of icy conglomerate nuclei with the compositions suggested above led immediately to reasonable jet forces and lags in emission for Encke's comet and others, requiring nuclei with radii of only a few km. The loss of matter needed to account for the non-gravitational forces was only a fraction of one per cent per revolution. For longer period comets the jet force radial towards the Sun should exceed the transverse lag component. This has since been demonstrated¹⁷ as well as non-gravitational motions among most of the periodic comets¹⁸.

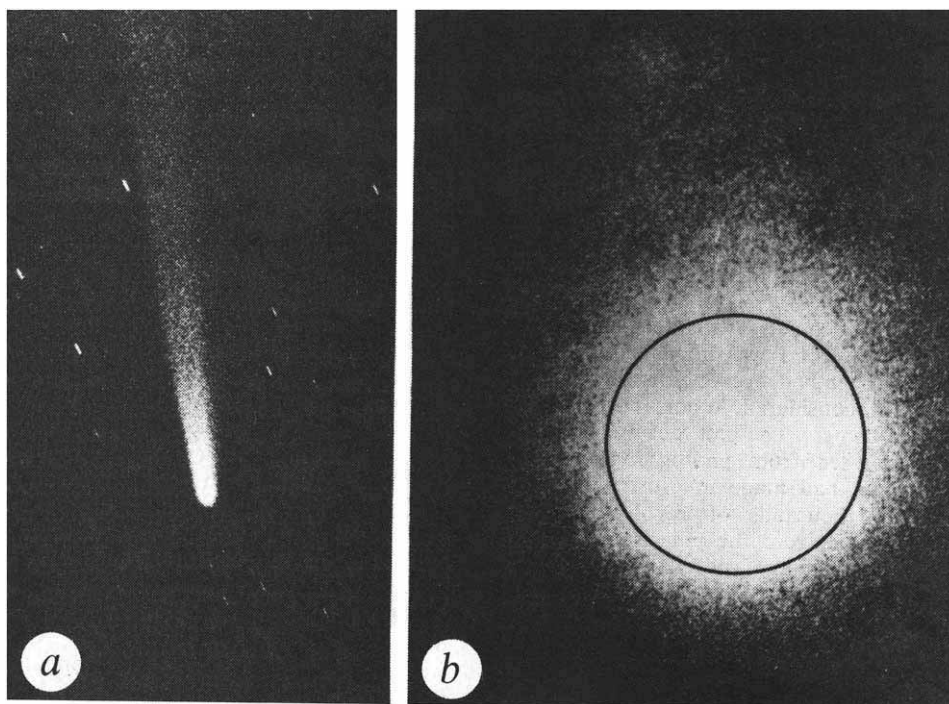


Fig. 1 Comet Kohoutek, 1973XII, Dec. 25.9, 1973. *a*, Photograph, Comet and Asteroid Observatory, South Baldy, New Mexico. *b*, NASA Skylab photograph in far-ultraviolet hydrogen $\text{Ly}\alpha$ radiation. Both photographs are on the same scale as is the Sun's disk (circle) at same projected distance.

The great loss of H_2O from comets to be expected from the icy model was explored by Biermann and Trefftz¹⁹ in 1960, who predicted a resultant huge hydrogen cloud observable by its Lyman α -emission line in the far ultraviolet (at $\lambda=1,416 \text{ \AA}$). The observations from space vehicles dramatically verified this prediction for Comet Bennett, 1970II (refs 19, 20). The hydrogen cloud about Comet Kohoutek, 1973XII, in Fig. 1 dwarfs our old ideas about the spatial extent of bright comets. The total loss of H_2O amounted to a significant fraction of a 1 km^3 , typical of brighter comets.

Even though Comet Kohoutek may have been a dud in the eyes of the public, it was a huge success scientifically because it was the best observed comet in all history. Radio observations in the millimetre range produced spectacular new results: the identification of hydrogen cyanide, HCN (ref. 22) and methyl cyanide, CH_3CN (ref. 23). Methane and ammonia still remained elusive but the H_2O positive ion was identified in the optical spectrum and atomic O and C in the far ultraviolet. The neutral H_2O molecule resisted discovery until Comet Bradfield, 1974III. Comet Kohoutek brought the theorists to recognise the complex possibilities of gas-phase chemistry in the head of a comet, which leaves uncertain the true identity of some parent molecules because some of the radicals may be split up or chemically changed in the cometary atmosphere.

The presence of chemically ill-mated carbon molecules and the apparent low abundance of CH_4 in Comet Kohoutek show clearly that comets are a mixture of low temperature compounds, not formed by slow cooling from a hot gas. Such carbon compounds are prevalent in interstellar gas so that their occurrence in comets suggests a similarity between comets and the interstellar medium. Most students of the subject believe that comets probably formed in the outer parts of the planetary system, but do not exclude direct contributions from the interstellar cloud that collapsed to form the Solar System, or even the bare possibility of comet accretion in fragmented interstellar clouds connected gravitationally with the Sun.

The brilliant dusty Comet West, 1975n, split into at least four components, giving us an intimate look at a discrete comet nucleus. Figure 2 presents five successive large scale photographs of the head made at the New Mexico State University with their 20-inch reflector. The components are behaving as would be expected for multibodied nuclei: they are separating! The relative velocities are small, however, only a few m s^{-1} . But more interesting are the brightness variations shown on these beautiful photographs. At times components D, B and C are as bright or brighter than component A. From an analysis of the motions, Sekanina²⁴ finds that component A is the main body of the comet moving in nearly a gravitational orbit. Component D separated first on February 13 starting at zero relative velocity with a relative deceleration, $d=2.85 \pm 0.03$ in units of 10^{-5} solar acceleration. Component B separated second with $d \sim 5$ and C third with $d \sim 40$, an order of magnitude greater deceleration than D and B. As Sekanina predicted, C was short lived, becoming unobservable after three weeks from its separation on March 5.

Note the remarkable paradox. Component C must surely have been very much smaller and less massive than component A because it disappeared rapidly and because it displayed a huge non-gravitational deceleration, indicating a very much larger area-mass ratio for jet action. Nevertheless, component C outshone component A on at least two occasions. An explanation of this phenomenon lies inherent in the icy comet model. Let us accept the concept that the interior of an active comet contains a significant fraction of materials much more volatile than water ice. The surface layers, vaporising in sunlight, must become stratified with non-volatile dirt on the surface, water ice below, and successively more volatile materials underneath. The rate of mass loss is tempered by the dirt and hard-to-vaporise water ice. An exposed inner surface, however, will become far more active because of the materials with low heats of vaporisation.

The key to our explanation now lies in the simplistic fact that a solid of any shape whatsoever, when broken in



March 24.5

March 18.5

March 14.5

March 12.5

March 8.5

1976

Fig. 2 Nucleus of Comet West, 1975n, on 5 dates. Component A, lower left; B, upper right; C, lower right; D, middle left. Scale on March 24.5: A to B = 16.3". Distance from Earth: 0.88 to 1.09 AU, March 5.5 to 24.5. From A. S. Murrell and C. F. Knuckles, New Mexico State University Observatory.

two pieces, exposes identical interior surface areas for each piece. Thus the small piece of a split comet nucleus exposes as much new surface containing highly volatile materials as does the large piece. In certain orientations with respect to the Sun it can produce more activity than the large piece and hence outshine it. Continued breakage of the smaller component undoubtedly adds to its activity, to its rapid demise, and to its high decelerating force by jet action. Thus Comet West demonstrated before our eyes one of the striking properties of a dirty-ice comet nucleus.

As to the cause of comet splitting, other than tidal disruption, we have no definite proof. Rapid rotation produced by asymmetric jet action with resultant spinup seems to provide a reasonable explanation. It is not yet certain whether the equatorial layers need be spun off against gravity or whether the weak gravity, perhaps reduced by rapid rotation, allows the underlying volatiles or pockets of volatiles to eject large volumes of surface. In any case, splitting begets more splitting as would be expected if rotation has a role. A body in space will eventually rotate about the axis with maximum moment of inertia, being in unstable rotation about other axes. Loss of material usually changes the distribution of inertia so that after a split, the body will seek a new axis of rotation and alter the internal forces. More splitting may be expected and is frequently observed.

Plasma tails present a fascinating complex problem area for theoretical study and, indeed, have become a kind of space laboratory for magnetohydrodynamics. When instrumented space probes finally reach comets, the cometary space laboratory will become a reality, to answer many of these physical problems, as well as those of gas-phase chemistry in comets and, most important, to tell us about the true composition and physical structure of comet nuclei. From space probes we can hope to pin-point the circumstances of cometary origin and their relation to other bodies in the Solar System, particularly the Earth. I am convinced that comets contributed significantly to the life-giving volatiles of the Earth and, indeed, would not be surprised to learn that material contributed by comets made possible our very existence on this planet.

I wish to thank my wife, Babette, for assistance in making this paper more intelligible to non-astronomers.

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articles

Transitions in double-diffusive convection

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The transitions of solutions of differential equations from one stability regime to another are of great current interest, both mathematical and physical, and there are conflicting hypotheses as to how such transitions occur. Here we present the results of an investigation of double-diffusive convection, which is important in oceanography, astrophysics and chemical engineering. The calculated transitions are found to be very different from those previously suggested.

Most fluid motion is turbulent. One approach that has been used in investigating this form of motion is to examine the transitions undergone by an initially laminar flow in its evolution to a turbulent state. Landau¹ hypothesised that as some appropriate non-dimensional parameter, R , increases, a critical value is reached at which the particular time-independent laminar flow under investigation becomes unstable and equilibrates to a new time-dependent flow. At a larger critical value this flow itself becomes unstable and this process of transitions is envisaged to continue until the flow has become so complicated that it would be referred to as turbulent. An alternative hypothesis has been recently suggested by Ruelle and Takens², whereby after a relatively small number of transitions, a well defined value of R is reached at which the flow exhibits an abrupt transition to a far more complicated, random, and hence turbulent, motion. Since these are both abstract hypotheses, only a direct investigation of the governing equations of motion can decide if either of these descriptions is correct, or if a different set of transitions takes place. In an investigation of this sort, but motivated by biological considerations, May³ recently determined the transitions that occur in a number of first-order nonlinear difference equations. He found that as the appropriate R increases through a sequence of critical values, the solution changes from consisting of one stable equilibrium point, through stable cycles of period 2^n ($n=1,2,\dots$), and then, at a finite value of R , there is chaos, with slightly different initial conditions leading to solutions which diverge with time. We present here the results of explicit calculations of the transitions that occur in two-dimensional double-diffusive convection. The motion is governed by a set of coupled nonlinear partial differential equations and the transitions that occur are different in form from the three discussed above.

Double-diffusive convection

Double-diffusive convection is a generic term for the type of convection that occurs in fluids in which there are two components of different molecular diffusivities which contribute in an opposing sense to the vertical density gradient. For different sets of components, this form of convection has an important role in oceanography, astrophysics and chemical engineering⁴. Here we use the terminology of heat and salt, the components appropriate to oceanography. We restrict attention to the

Rayleigh-Bénard problem⁴, where the fluid is considered to occupy the space between two infinite planes separated by a distance D , with the upper plane maintained at temperature T_0 and salinity S_0 and the lower plane maintained at temperature $T_0 + \Delta T$ and salinity $S_0 + \Delta S$ ($\Delta T, \Delta S > 0$). We assume both planes to be stress free and perfect conductors of heat and salt, and restrict attention to two-dimensional motion, dependent only on one horizontal coordinate and the vertical coordinate. Expressing all lengths in units of D , time in units of D^2/κ_T (where κ_T is the thermal diffusivity) and representing the temperature T^* and salinity S^* by

$$T^* = T_0 + \Delta T(1 - z + T); \quad S^* = S_0 + \Delta S(1 - z + S)$$

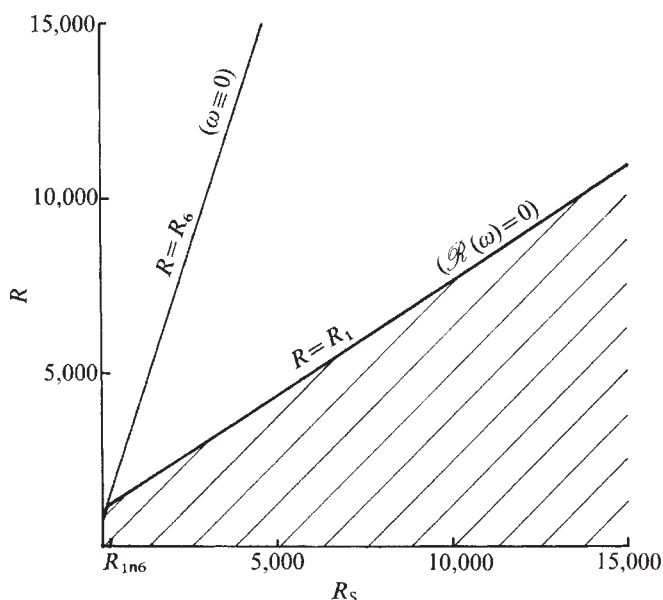
we can write the governing Boussinesq equations of motion in terms of a stream function ψ as

$$\begin{aligned} \sigma^{-1} \nabla^2 \partial_t \psi - \sigma^{-1} J(\psi, \nabla^2 \psi) &= -R \partial_x T + R_S \partial_x S + \nabla^4 \psi \\ \partial_t T + \partial_x \psi - J(\psi, T) &= \nabla^2 T \\ \partial_t S + \partial_x \psi - J(\psi, S) &= \tau \nabla^2 S \\ \psi = \partial_{zz}^2 \psi = T = S = 0 \quad (z = (0,1)) \end{aligned}$$

where

$$J(f, g) = \partial_x f \partial_z g - \partial_z f \partial_x g$$

Fig. 1 The results of linear stability analysis for $\sigma = 1$ and $\tau = 10^{-1}$. Overstability first occurs along $R = R_1$ and monotonic stability first occurs along $R = R_6$. Only the hatched region is stable to linear disturbances.



The four non-dimensional parameters appearing in these equations are: the Prandtl number $\sigma = \nu/\kappa_T$, where ν is the kinematic viscosity; the ratio of the diffusivities $\tau = \kappa_S/\kappa_T$, where κ_S , the saline diffusivity, is less than κ_T ; the thermal Rayleigh number $R = \alpha g \Delta T D^3 / \kappa_T \nu$, where α is the coefficient of thermal expansion, and g is the gravitational acceleration; and the saline Rayleigh number $R_S = \beta g \Delta S D^3 / \kappa_T \nu$ where β is the saline analogue of α .

Stability

The criteria for the linear instability of these equations are obtained by neglecting the nonlinear Jacobian terms and representing the solutions in terms of the lowest normal modes with an exponential time dependence of the form $\exp(\omega t)$. The onset of overstability, defined by $\Re(\omega) = 0$, first occurs for a value of R which we denote by R_1 and exchange of stabilities, defined by $\omega \equiv 0$, first occurs for a value of R which we denote for convenience by R_6 . In both cases the horizontal wavelength of the instability is $2^{3/2}$. In the R - R_S plane the linear stability boundary is a combination of $R = R_1$ and $R = R_6$, as depicted in Fig. 1, which presents a summary of the linearised results for $\sigma = 1$ and $\tau = 10^{-1/2}$. For $R_S > R_{106}$, where R_{106} is the value of R_S at which $R_1 = R_6$, as R exceeds R_1 the conduction state ($\psi = T = S = 0$) becomes unstable to an oscillatory mode. For $0 < R < R_{106}$ as R exceeds R_6 the conduction state becomes unstable to a monotonic mode.

We present here the form of the solutions of horizontal wavelength $2^{3/2}$ which emanate from the above linear transitions, or bifurcation points, as the degree of nonlinearity increases. An appropriate way to describe the resulting solutions is as a function of their amplitude. This is conveniently represented by either the thermal or saline Nusselt numbers evaluated by the lower boundary

$$N_T = 1 - \overline{\partial_z T}|_{z=0} \quad \text{and} \quad N_S = 1 - \overline{\partial_z S}|_{z=0}$$

where the overbar denotes a horizontal average, or by their respective temporal maxima, M_T and M_S . Since for most physical systems $R_S > R_{106}$ and this is conceptually the most interesting case, we concentrate on the latter regime and, principally by numerical integration of equations (1), map out the forms of equilibrium solutions in an R - M_S plane in the manner depicted in Fig. 2. The discussion is in general terms; specific details and physical interpretations of the solutions will be published elsewhere (H. E. Huppert and D. R. Moore, in preparation).

The bifurcation point at R_1 can be either supercritical (R increases as M_T or M_S increases) or subcritical (R decreases as M_T or M_S increases). By the straightforward use of modified perturbation theory⁵, a very lengthy relationship can be determined (H. E. Huppert and D. R. Moore, in preparation) which indicates, for fixed σ , τ and R_S , which of the two possibilities occurs. It is known from general theory⁵ that solutions on branches emanating from subcritical bifurcations are unstable until the branch reaches a minimum value of R . Thereafter the branch continues with the amplitude of the associated time-dependent solutions increasing with increasing R . Supercritical branches are known to support stable solutions. A typical plot of N_T and N_S against time for a solution on a stable portion of the branch sufficiently close to R_1 is shown in Fig. 3a.

Increasing R

As R increases, this form of motion continues until R reaches a specific value, R_2 say. At $R = R_2$ the solution changes in form and develops a further structure as is indicated in the form of N_T or N_S as a function of time, as plotted in Fig. 3b. In both N_T and N_S there are four extrema, two maxima and two minima, per period, where the period is defined in the usual sense as the time between two identical states. In modern mathematical jargon, the solution for $R < R_2$ is on a sphere, while the solution

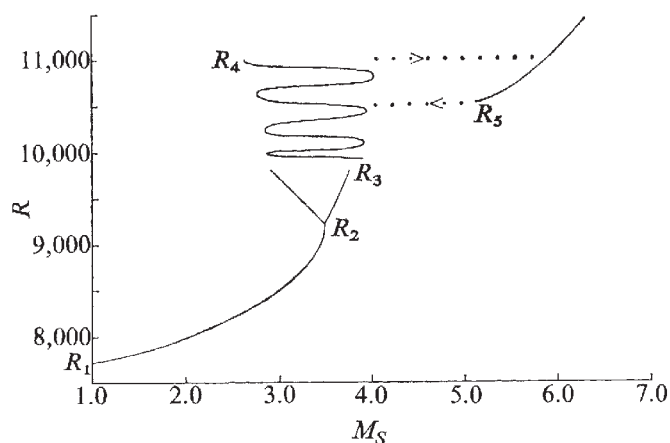
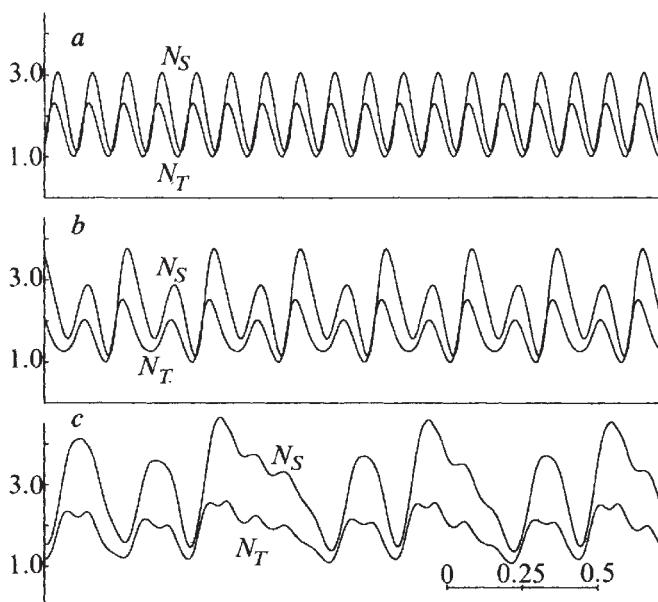


Fig. 2 The stable oscillatory branch and the stable monotonic branch for $\sigma = 1$, $\tau = 10^{-1/2}$ and $R_S = 10^4$. For $R_2 < R < R_3$ the branch is drawn so as to show both local maxima. For $R_3 < R < R_4$ the wavy line indicates that the solution is non-periodic and no definite maximum can be assigned. The dots at $R = R_4$ and $R = R_5$ indicate the jumps in M_S which occur as the solution changes from the oscillatory branch to the monotonic branch.

for $R > R_2$ is on a torus, and the transition at $R = R_2$ is called a bifurcating torus⁶. This form of motion continues until $R = R_3$ say, at which value a transition to a disordered solution occurs. A typical plot of N_T and N_S against time for such a disordered solution is shown in Fig. 3c. Long computation runs have not revealed any discernable periodic structure in the solution. Such non-periodic solutions continue to exist for increasing R until, for $R = R_4$ say, the only equilibrium solutions are time independent. For some values of σ , τ and R_S the transition at R_4 occurs before the one at either R_2 or R_3 .

For all $R > R_4$ all equilibrium solutions are independent of time. Time-independent motions are more efficient at transporting heat and salt than time-dependent motions and thus the Nusselt numbers undergo a discontinuous increase as the solution changes from being on the oscillatory branch to the monotonic branch, as indicated in Fig. 2. As R increases

Fig. 3 The thermal and saline Nusselt numbers as a function of time for $\sigma = 1$, $\tau = 10^{-1/2}$ and $R_S = 10^4$. a, $R_1 < R = 8,600 < R_2$; b, $R_2 < R = 9,800 < R_3$; and c, $R_3 < R = 11,000$. The horizontal line in the bottom right-hand portion of c, represents non-dimensional time.



beyond R_4 the effects of the salt field decrease, and for $R \gg R_4$ the equilibrium solutions approach those for $R_S = 0$, a situation which has been intensively investigated by Moore and Weiss⁷.

Decreasing R

If R is gradually decreased from some value greater than R_4 , the equilibrium monotonic solutions retrace the states that would have been obtained on increasing R from R_4 ; for each $R > R_4$ there is a unique stable equilibrium solution. If R is decreased below R_4 , an equilibrium time-independent solution continues to exist, with decreasing amplitude, until $R = R_5$ say. Further decrease of R leads to a solution on the oscillatory branch already described, or if $R_5 < R_1$ to the null solution. Thus, as indicated in Fig. 2 there is hysteresis between the two different forms of solution.

The time-independent branch of solutions emanates from the bifurcation point at $R = R_6$, which modified perturbation theory shows to be subcritical if $R_S > R_{106}$. As mentioned previously, solutions on such a subcritical branch are unstable until the minimum value of R is attained. Thereafter the branch continues and solutions on it are stable, with the amplitude of the solution increasing with increasing R .

If $R_S < R_{106}$ only time-independent equilibrium solutions are possible. For sufficiently small R_S the bifurcation at R_6 is supercritical, otherwise it is subcritical (H. E. Huppert and D. R. Moore, in preparation).

Conclusion

To summarise, in the most general case, as R increases there is a transition from the conduction state to an oscillatory motion (Fig. 3a), followed by a transition to a more complicated form of oscillatory motion (Fig. 3b), followed by a transition to a non-periodic, random state (Fig. 3c), followed by a transition to steady motion. Hence by increasing R it is possible in this situation to suppress disordered motions. For some values of σ , τ and R_S a sufficiently large disturbance can cause a transition directly from the conduction state to the steady state, while for other values of σ , τ and R_S only some of the intermediate transitions are omitted.

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Stratified waters as a key to the past

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Density stratification in lakes and oceans generate anoxic conditions below the pycnocline, and sediment facies mirror this development. A comparison of modern sediments deposited in stratified and non-stratified waters with sediments formed since the Cambrian reveals that the ancient sea has been stratified a number of times.

THE world ocean is fully oxygenated except for some local areas in regions of upwelling or stagnation. Oxygenation is related to modern climates, that is thermo- and haloclines, which can inhibit the mixing of water masses between stratified layers but which are only temporarily stable. Furthermore, cold polar surface water sinks and moves towards the equatorial abyssal plains. These and other climate-controlled physical factors turn over the ocean's water in a matter of a few hundred to a few thousand years. At this rate, molecular oxygen is recharged much faster in the deep sea than it is consumed by the oxidation of organic matter at greater water depths.

During prolonged warmer climatic stages or when landlocked seas develop, thermo- or haloclines may become so stabilised that they only move up and down in response to seasonal changes, tectonic activities, or some other major perturbations in the environment; but they rarely break up entirely. In such conditions molecular oxygen will remain abundant in the euphotic zone but will gradually drop to zero below the density boundary. This will cause the development of a euxinic environment in which no higher forms of life can exist and molecular oxygen is replaced by hydrogen sulphide.

It is important to know what happens to the strata when this

situation arises because such information is crucial to the task of explaining the origin of euxinic sediments, which are so plentiful in the stratigraphic record. Unfortunately, comparative studies between shallow and deep-water habitats are hindered because most of the marine sediments exposed on continents are of shallow-water origin and suites of abyssal sediments have only recently become available through the Deep Sea Drilling Project.

A significant aspect of this problem which has received little attention in the past concerns a possible feedback mechanism between oxidising and reducing environments which may result in the formation of specific sediment types.

We will focus attention here on the feedback question by examining sediments of the same ages and geological settings which differ only in that one group has formed below and the other above a well defined thermo-halocline. The sediments are from cores from the Black Sea and some deep East African rift lakes and they represent continuous sections through parts of the Holocene and Pleistocene. A detailed examination of a thermo-halocline, oscillating through time, will allow us to follow in slow motion the impact of the reducing on the oxidising environment and vice versa.

Phase boundaries

An heuristic theorem implies that physicochemical phenomena established at the boundary between two different states characterise these two states. In the present context the theorem implies that the physicochemical properties of the reducing and oxidising environments are 'written' on the boundary layer, that is, the thermo-halocline. We will now examine this interface.

Mechanism and rate of molecular exchange across a well

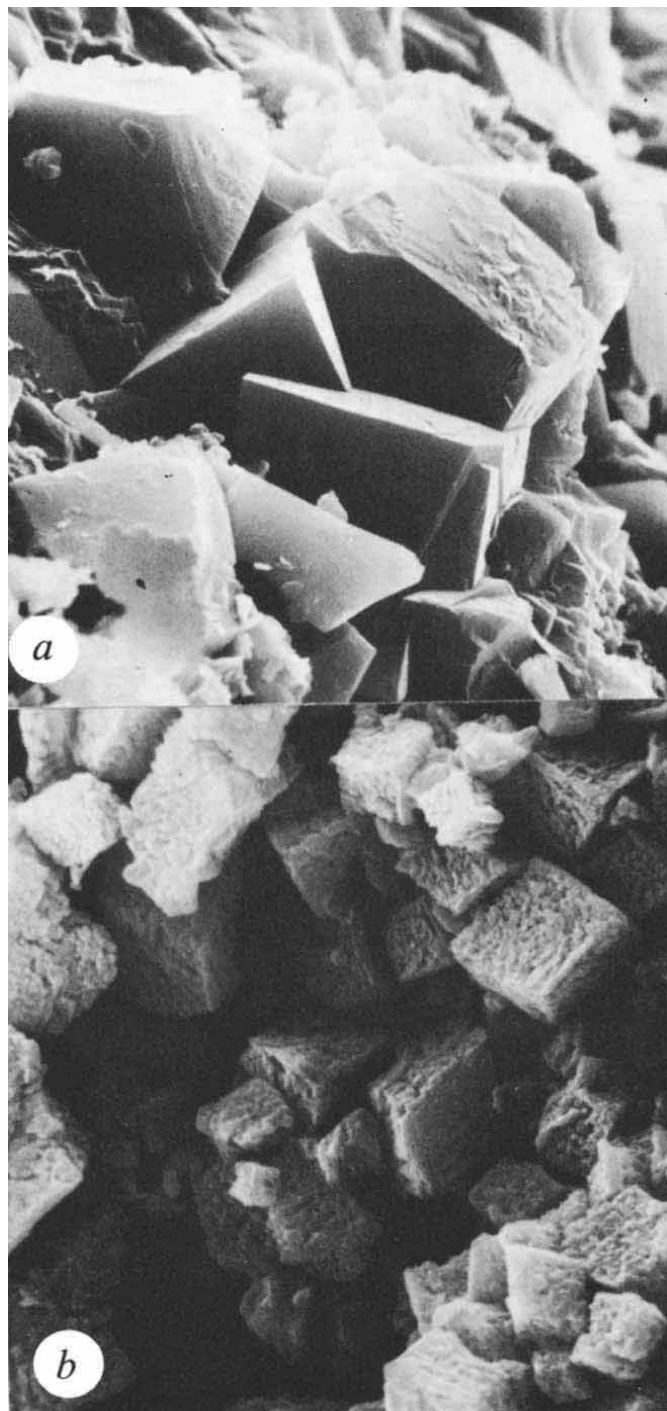


Fig. 1 Scanning electron micrographs of chemically precipitated calcites, sedimented above O_2 - H_2S interface (a), and below interface (b). Size of individual crystals ~ 5 - $10\ \mu m$.

developed thermo-halocline have been studied thoroughly¹⁻³. Vertical advection and diffusion will move deep water through the density boundary to the surface layer. For the main pycnocline in the Black Sea this results in a vertical eddy-diffusion coefficient of $\sim 0.014\ cm^2\ s^{-1}$ (refs 2 and 3). From this value one can readily calculate the net upward flux of dissolved species such as iron, manganese, or hydrogen sulphide. Against the upward advective gradient there is a downward diffusion gradient of oxygen. These two opposing fluxes will generate a wide range in redox potentials within a narrow segment of the water column. Some elements and minerals will respond to this *Eh* gradient by becoming reduced or oxidised, and precipitated or dissolved, respectively. In turn, minerals can form at one point, but by sinking through an *Eh* gradient they may

redissolve. Depending on the speed of this reaction and the general hydrographic setting, the process of precipitation and dissolution can continue again and again and lead to substantial concentrations of certain elements and minerals close to the pycnocline.

Models using a constant eddy-diffusion coefficient for the vertical transfer of passive properties in a continuously layered medium^{4,5} are only applicable for periods of hours and days because density boundaries tend to rise and fall and many other physical perturbations do exist⁶⁻⁹. If the interface is lowered rapidly, an almost spasmodic mineralisation of the upper layer will take place resulting in massive precipitation of a series of minerals. In contrast, a rise of the interface will dissolve the same minerals. Figure 1 shows calcite which has precipitated in the upper layer. In Fig. 1a the calcite remained in the surface layer, whereas in Fig. 1b it sank into the anoxic water. It is of note that all carbonates including dolomite and siderite will eventually dissolve if exposed to highly reducing environments.

East African rift lakes

The deep rift lakes of East Africa—Tanganyika, Malawi and Kivu—are thermally stratified. The perennial thermocline in Lake Tanganyika lies at $\sim 100\ m$ but is somewhat deeper and much more variable in Lake Malawi. Lake Kivu has a stable thermo-haline density structure with an interface at $\sim 70\ m$ (ref. 10). The position and nature of the density boundary influence the occurrence of distinct mineral and chemical facies observed in sediment cores of the three lakes¹⁰⁻¹³. Most notably affected by the position of the interface is the distribution of Fe-oxides, Fe-silicates, Fe-phosphates, Fe-sulphides, Mn-siderites and a series of Ca- and Ca-Mg-carbonates. In the following, we will examine sediments from deep ($\sim 500\ m$) and shallow ($\sim 50\ m$) parts of Lake Kivu that were deposited during the past 10,000-14,000 yr.

The impact of an oscillating stratification is illustrated by the distribution of manganese and related facies in a core from the central basin (see Fig. 2). The observed Mn^{2+} oscillations are interpreted as resulting from the following processes¹⁴. Thermal stratification allows Mn^{2+} and Fe^{2+} to accumulate in the water of relatively low *pH* below the thermocline. Vertical advection and diffusion transfer both ions through the interface where they instantaneously precipitate as manganosiderite. The newly formed mineral phase sinks back into deep water, dissolves, and the cycle starts over again. Its incorporation into the sediments will only be possible near the thermocline. The manganosiderite in Lake Kivu occurs, however, as discrete layers rather than as a mixture with the regular detritus. This suggests a more powerful transfer mechanism than advective eddy-diffusion: the rapid lowering of the thermocline and a resultant massive precipitation of the manganosiderite. Fluctuations in Mn^{2+} content shown in Fig. 2 reflect oscillations of the thermocline which shifted the area of manganosiderite deposition away from or back towards the location of the core site. High water stands are indicated by the emergence of sulphides and specific diatom assemblages in an organic-rich facies free of carbonates^{14,15}.

The profile continues in Fig. 3. This time, however, carbonate and organic carbon serve as indicators for the oscillating O_2 - H_2S boundary¹⁰. Carbonate precipitation starts with high Mg-calcites and the percentage of $MgCO_3$ in solid solution is indicated numerically. At $CaCO_3$ concentrations $> 10\%$, aragonite occurs. A change in mineralogy from Mg-calcite to aragonite reflects an increase in the Mg-Ca ratio in the lake water¹³ which can best be explained by progressive evaporation which rapidly will lower both the interface and the water level and enforce lime extraction. The emergence of carbonate-free sapropels signals meromixis. Surface waters have *pH* values between 8 and 10 but as the *pH* drops below 7 the waters become anoxic. Carbonate particles falling into the deep water redissolve, whereas those that settle in shallow areas above the pycnocline remain intact and form thick sediment sections. Changes of the Mg-Ca ratio in the lake water will

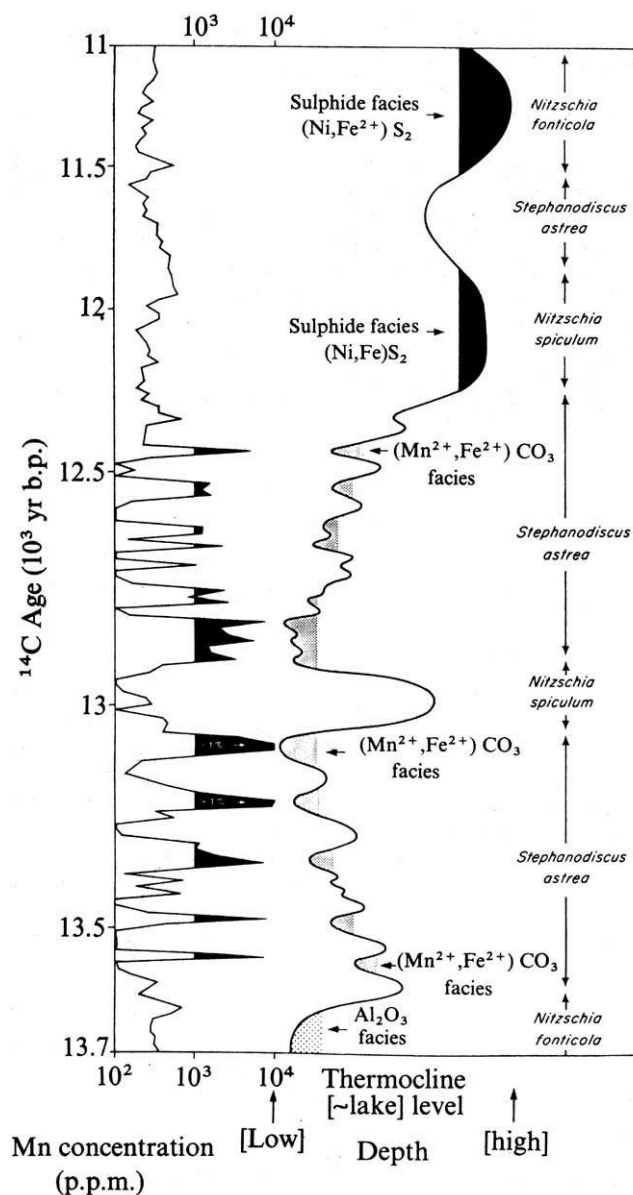


Fig. 2 Distribution of manganese in Kivu sediments deposited during the time interval between 13,700 and 11,000 yr BP. Mineral facies and reconstructed thermocline oscillations are shown. High water stands are indicated by the emergence of sulphides and specific diatom assemblages^{14,15}.

determine the carbonate mineralogy^{13,16}. Calcite, Mg-calcite, aragonite, protodolomite and monohydrocalcite generate cyclic patterns in the shallow basins of Lake Kivu, while at the same time a thin sapropel facies forms in the deep anoxic basin of the lake. Similar relationships exist in Lake Tanganyika^{11,13}.

The impact of an oscillating density boundary or a 'catastrophic' overturn is registered by the established fauna and flora. For instance in Lake Kivu the fish fauna is depauperate, chaoborids are absent and the molluscs, especially pelecypods, have suffered recent extinctions, judging from the abundance of shells of species which are no longer extant in the lake^{15,17}. Particularly illuminating is the distribution pattern of diatoms over the past 14,000 yr (refs 14, 15 and 18). *Stephanodiscus astrea* declines almost linearly from ~ 90% of the total diatom population at ~ 14,000 yr BP to 0 at ~ 5,000 yr BP. Similarly, *Nitzschia fonticola* disappears at ~ 5,000 yr BP. Only one species, *Nitzschia spiculum*, crosses this boundary and is joined by newly emerging diatom species. These and related observations strongly suggest that changes in water chemistry introduced by a fluctuating interface can severely disturb the

established ecosystem. Whole populations can be exterminated; others can be pushed into restricted ecological niches from where they may perhaps reoccupy their former territory should the habitat change back to its former condition. Some species may adapt to the new environmental circumstances, whereas others may take advantage of the new situation and become dominant. In addition, some new species may evolve.

Lake Tanganyika, which has had a series of oscillating thermoclines and water levels¹⁹⁻²², is a good test case for the evolution of species in a closed system. A large number of endemic genera and species is present in the modern lake²³, especially fish. When counted in 1960 (ref. 24), *Cichlidae* alone numbered no less than 133 species from 38 genera. Another point of interest is the striking resemblance of a great variety of present-day snails to certain marine forms that became extinct in the Jurassic^{25,26}. All this has to be viewed against a background of a lake which formed in Miocene time and has never been open to the sea. In conclusion, the antiquity of Lake Tanganyika is mainly responsible for the evolutionary diversification, but temporal variability in lake levels, water chemistry, and stratification are considered dominant factors in triggering this development^{14,15}.

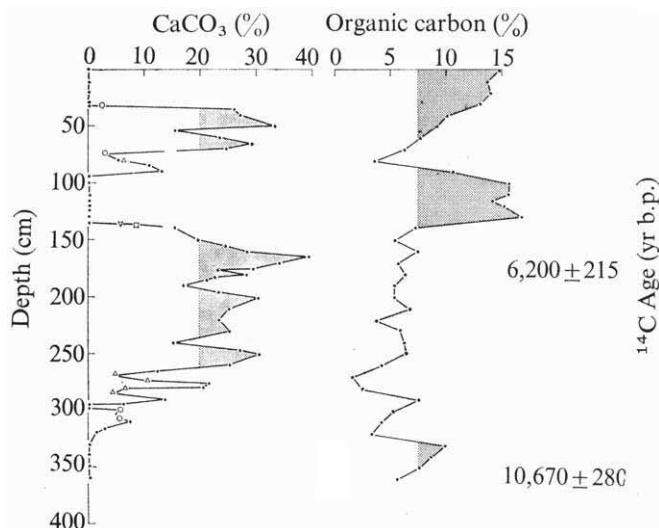
Black Sea

Much has been learned about the recent history of the Black Sea through a R/V Atlantis research cruise conducted by the Woods Hole Oceanographic Institution in spring 1969 (ref. 27). A topic of special significance for our work is the evolution of anoxic conditions during the Holocene²⁸.

Each gram-atom of carbon in plankton requires 1.3 mol of oxygen for its oxidation²⁹. Since the amount of organic matter that falls through the O₂-H₂S interface is fairly well known³⁰, the 'decay' constant of the oxygen reservoir can be calculated²⁸. From this number, the upward progression of the O₂-H₂S interface can be derived. Calculations show that the thermohaline boundary requires ~ 3,000 yr to rise from the 2,200-m-deep abyssal plain to a water depth of 500 m. Varve-counting techniques substantiate these findings³¹ and indicate that finely laminated sapropels began to occur on the basin slope at a depth of 470 m almost 2,300 yr after they first appeared on the bottom of the Black Sea basin.

The sapropel facies is low in carbonates and many grains show corrosional features of the kind shown in Fig. 4. Of special note is the sapropel facies in a core from a water depth of 470 m which rests on a massive calcareous mud and is

Fig. 3 Distribution of CaCO₃ and organic carbon in Kivu sediments deposited during the past 11,000 yr (continuation of profile shown in Fig. 2). Numbers on carbonate curve represent the mole percentage of MgCO₃ present in high Mg-calcite; water depth: 473 m (ref. 10).



characterised by intercalations of very fine authigenic carbonate laminae up to 2 mm thick at the base of the sapropel but becoming finer and eventually disappearing towards the top of the sapropel layer. All these observations fit the general pattern described for Kivu sediments, and therefore indicate the same mechanism of formation.

Present—a key to the past

We have studied stratified waters in numerous environments for more than ten years^{10–15, 27, 31, 32}. It never occurred to us, however, that the results of these studies could significantly alter established concepts in geology. In other words, the subject was looked at more as an interesting anomaly rather than as a feature of global significance.

This outlook changed when we described sediment cores from the Deep Sea Drilling Project's Leg 42 B obtained by the Glomar Challenger in the Black Sea during May–June 1975. In three cores from the euxinic abyssal plain (core length 624.5 m) the basin apron (core length 1,073.5 m) and the basin slope (core length 503.5 m) a kind of textbook profile emerged consisting of successions of (1) shallow- and deep-water facies, (2) flysch and molasse cycles, (3) oxidising and reducing sediments, (4) clastic and chemical deposits, and (5) freshwater–brackish–marine series. Most striking is the distribution of authigenic carbonates. In going from the shallow to the deep basin, a typical facies succession is dolomite–aragonite–high magnesium calcite–calcite–siderite–carbonate–free sapropels. In view of these results, we will briefly compare present and past common sediment facies of the open marine environment. Since authigenic carbonates appear to be excellent environmental indicators, we will use them as tools in our comparison.

In modern marine environments, the chemical precipitation of calcium carbonates or dolomites is virtually nil. Recent marine carbonates are principally biological in origin. Calcareous forams and coccolith oozes cover wide parts of the deep sea and coral reefs, and coquina are found in some shallow-water environments. The distribution of calcareous oozes is controlled by the local carbonate compensation depth and a few other critical factors determining rates of dissolution³³. In contrast, sand and clay are the typical facies in modern shallow-water deposits.

The stratigraphic record, from the Cambrian on, indicates an almost reversed situation. Hundreds of metres of shallow-water carbonates, distributed over extensive areas, are frequently encountered. Sedimentary cycles measuring not more than a few centimetres or metres can be traced over wide distances. On the other hand, an ordinary geosyncline commonly starts with a massive graywacke facies or a black shale facies free of carbonates. Judging by their physical appearance and chemistry, these sediments formed at great water depths and under anoxic conditions. In conclusion, sediment facies in the Black Sea and some East African rift lakes have many characteristics in common with ancient marine sediments, whereas sediment facies in the present ocean show little resemblance to the past record. These findings have interesting consequences because they suggest that the former ocean looked much like the present Black Sea. Two simple graphs will summarise the most critical points.

In the modern fully oxygenated ocean, sands and clays are deposited in the shallow sea (Fig. 5a). On stratification of the water, anoxic conditions develop below the interface and euxinic sediments form in the H_2S zone, whereas carbonates may develop in the oxic zone (Fig. 5b). It is essential to know that these two contrasting environments not only differ in oxygen and hydrogen sulphide but in a number of other chemical ingredients. As a common rule the anoxic waters are enriched in (1) mineral nutrients such as nitrate, phosphate, ammonia and silica, (2) some common elements, and (3) dissolved gases.

Lowering of the interface (Fig. 5c) will result in the transfer of dissolved chemical species into the upper layer. Primary productivity will be stimulated and carbonates will precipitate with the Mg–Ca ratio in the water phase controlling the carbonate

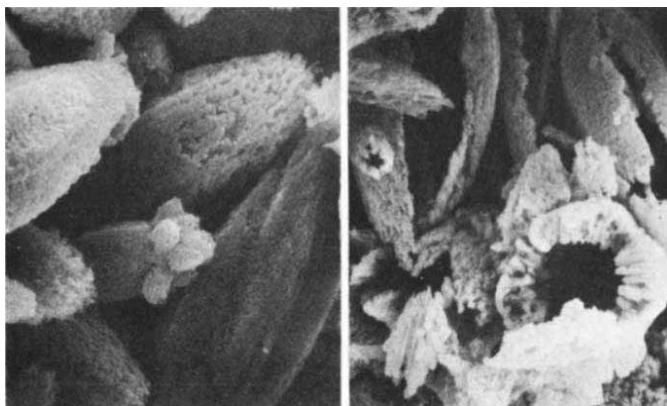


Fig. 4 Scanning electron micrographs of chemically precipitated aragonites buried below the O_2 – H_2S interface. Note dissolution features similar to Fig. 1b. Size of aragonite grains $\sim 20 \mu m$.

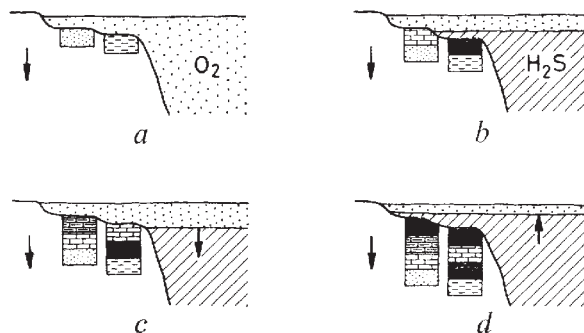
mineralogy. An upward progression of the interface (Fig. 5d) will extend euxinic conditions to shallower parts of a basin, with established benthic communities becoming extinct, and carbonates starting to dissolve because the level of carbonate compensation in time will be identical with the level of the O_2 – H_2S interface. As a function of climatic variation and constant reshaping of marine basins by tectonic or erosional forces, all transitions between a fully oxygenated and an anoxic sea are conceivable.

Sediment facies in the shallow water environment will mirror these events through distinct cycles and fossil assemblages. At O_2 times, the sediment distribution in the deep sea will resemble that of today, whereas at H_2S times, sapropels will occur, and calcareous oozes and marls will show pulses of dissolution. This process can go so far that a hiatus will develop and the dissolved chemical species will accumulate in the deep waters. Submarine volcanic activity and rifting will enhance these actions. It is of note that brines can accumulate in the deepest part of a basin. Upwelling or overturning of water masses will cause the release of these substances, and phosphorites, carbonates, and Fe–Mn oxides and hydroxides are a few of the major phases that may come into existence.

A graph (Fig. 6) shows schematically the pathway of four critical elements through geological time. The modern concentration level for each of the four elements in the deep sea is shown at the far right. The main features are as follows:

(1) Fe^{2+} becomes oxidised in the euphotic zone. Lowering of the O_2 – H_2S interface will release Fe^{2+} to the upper layer causing the formation of siderite, a series of Fe-oxides and hydroxides, and Fe-silicates³⁴. In the anoxic sea, Fe^{2+} is extracted as sulphide or silicate (for example, chlorite). Towards the end of the Precambrian, the ocean water turned over, fully oxygenating the deep sea for the first time, but went back to the anoxic state during the Silurian. This sequence is repeated several times throughout geological time.

Fig. 5 Formation and evolution of stratified waters (see text).



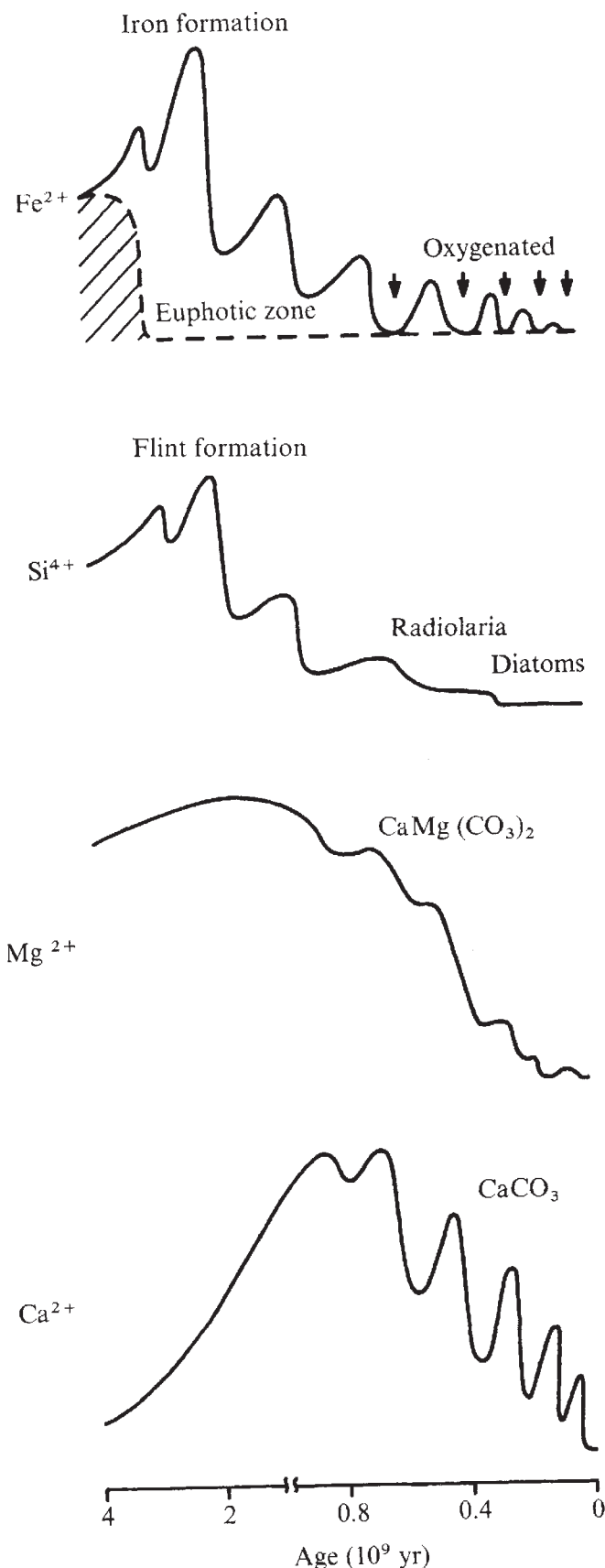


Fig. 6 Chemical evolution curves of Fe^{2+} , Si^{4+} , Mg^{2+} , and Ca^{2+} levels in the deep sea (schematic). The Fe^{2+} content in the upper layer drops to zero early in the Precambrian as soon as O_2 -generation reaches a critical level.

(2) In the Precambrian, the water is nearly saturated with silica below the interface. When released to the upper layer it precipitates on evaporation in shallow-water environments as amorphous silica. Biogenic silica extraction is the dominant release mechanism in more recent times.

(3) Mg^{2+} is extracted in the deep and shallow sea as silicate. Conditions for dolomite formation gradually develop and massive primary dolomite formation proceeds during the Palaeozoic and Mesozoic.

(4) Ca-carbonate deposition is dependent on the Mg-Ca ratio in the water phase¹⁸. Aragonite is the principal CaCO_3 species at the start of carbonate extraction, and high Mg-calcite, and eventually calcite, follow. Because of the instability of aragonite and high Mg-calcite, recrystallisation in the direction of hard dolomites and limestones is commonly observed in the older strata. In contrast, sediments composed of calcitic forams and/or coccoliths have a tendency to remain unindurated.

Conclusions

The highlight of the present study is the observation that permanently stratified modern marine and freshwater basins induce carbonate precipitation in the upper layer and carbonate dissolution in the anoxic deeper water. Vertical advection and diffusion will recycle dissolved chemical species back into the surface layer; however, a more substantial mineralisation of the euphotic zone is achieved by lowering the O_2 - H_2S interface. This will promote massive carbonate precipitation. As a result sediments above a density boundary get rich in carbonates while those beneath have little or none. Because climate and/or geological settings change, thermo- and haloclines move up and down the water column or break up entirely, disrupting established ecosystems. The sediment record will reflect these incidents in the form of distinct cycles, facies, fossil assemblages, or hiatus.

Hutton's principle of uniformity of process, which holds that the present is the key to the past, gets an unexpected tenor. The well mixed oxygenated ocean of today seems not to be the model environment for the past 600 Myr. Instead, we are dealing with a sea which alternates between two opposing states: stratified and non-stratified. Depending on local or regional hydrographic settings, which may effect deep-water circulation and the hydrodynamics of water movement vertically and horizontally, one may encounter all sorts of transitions. At present, some areas in the open ocean are already anoxic, and indicate the delicate balance between the oxic and anoxic states. All these findings lead us to conclude that a number of former interpretations regarding the origin of sedimentary cycles, the history of the oceans, and the workings of evolution have to be re-examined.

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Similarities and differences in the structure of X and Y chromosome rRNA genes of *Drosophila*

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In Drosophila melanogaster, the rRNA genes (rDNA) are clustered at single sites on two non-homologous chromosomes, the X and Y. Examination of the structure of X and Y rDNAs with restriction endonucleases reveals that the X rDNA contains repeating units not present in the Y. Such observations, as well as genetic evidence, illustrate difficulties with the hypothesis that recombination is the predominant mechanism preserving similarities between these two clusters maintained on different chromosomes. This raises the possibility that selection pressure has a significant role in maintaining the parallel evolution of these two separate but homologous redundant gene clusters.

In the fruit fly, *Drosophila melanogaster*, there is one nucleolus organiser (NO) on the X chromosome and one on the Y. At each NO, there are 200–250 tandemly arranged ribosomal RNA genes (rDNA)^{1,2}. Although these genes reside on non-homologous chromosomes, the 18S and 28S RNA molecules transcribed from either the X or Y rDNA have very similar, and probably identical, primary sequences as revealed by fingerprinting experiments.³ Furthermore, in the wild-type *D. melanogaster* male, which is genotypically XY, there is no meiotic recombination⁴ though mitotic recombination occurs in both both sexes⁵. Thus not only are the rRNA genes located on non-homologous chromosomes but they also seem to be genetically isolated from one another by virtue of the absence of meiotic recombination.

Attempts to elucidate the detailed molecular structure of *Drosophila* rDNA by electron microscopic analysis of actively transcribing clusters of rRNA genes have produced conflicting results. Hamkalo *et al.*⁶ found a very heterogeneous rDNA unit repeat length when embryonic material was used. Using similar techniques, Laird and Chooi (personal communication) have found that two length classes of rDNA repeats exist, both of which occur on the X NO, but only the shorter one was observed on the Y NO. Observations on rDNA fragments produced by the restriction endonuclease *EcoRI* and cloned in *Escherichia coli* revealed two distinct size classes of repeating units, one 17 kb (kilobases or kilobase pairs)⁷ and the other, 11–12 kb (D. M. Glover and D. S. Hogness, personal communication). We report here the distribution of these size classes on the X and Y NO. Our results define the restriction endonuclease products of the X and Y rDNA, and demonstrate that both NOs are similar with respect to one major class of repeating

elements but differ decisively in their content of another class of repeats.

Restriction endonuclease analysis of X and Y chromosome rDNA

On treatment of unfractionated DNA of appropriate genetic origin with *EcoRI* the fragments were separated by Agarose gel electrophoresis and transferred to nitrocellulose filters according to the method of Southern⁸. The filter-bound fragments were hybridised to radioactively-labelled rRNA and finally made visible by autoradiography. Figure 1a shows the results of a series of experiments in which females (X/X) from two different wild-type strains (Ore-R and Lausanne) and males carrying only the Y chromosome NO are compared. It is obvious that the X chromosome contains both 17- and 11-kb segments while the Y contains the 11-kb but not the 17-kb fragment. In addition to these bands, two components of about 7.4 kb and 5.4 kb occur in both X and Y NOs. A mixture of X and Y DNA digested with *EcoRI* also displays these four bands, indicating that there is no apparent difference in the size of *EcoRI* fragments derived from X or Y chromosomes. When purified rDNA is digested with *EcoRI* there are, in addition to the predominant 17-, 11-, 7- and 5-kb size classes, minor fragment classes of intermediate length, some of which can be seen as bands in Fig. 1b. Somewhat similar length heterogeneities have been observed among *Xenopus* rDNA repeats¹².

From mapping studies it is known that the 11-kb and 17-kb fragments represent complete repeating units of the rDNA, which are distinguishable by the insertion of an additional spacer sequence within the 28S gene region (Glover and Hogness, personal communication; unpublished results of P. K. Wellauer and I.B.D.). The nature of the shorter 5–7-kb fragments is not yet analysed, but it seems likely that they arise from the presence of additional *EcoRI* sites in a fraction of the repeating units. The other minor bands are due to additional *EcoRI* sites and to the length heterogeneity in the repeating unit.

The difference between the X and Y NOs can be demonstrated by other means. The restriction enzyme from *Serratia marcescens*, *SmaI*, yields a different fragment pattern from the two types of rDNA (Fig. 2). Y chromosome rDNA yields one fragment about 8.5 kb long and a second fragment 2.8 kb long, both of which hybridise to 28S and 18S RNA. *SmaI* digestion of X rDNA, however, produces a further fragment of 2.2 kb which hybridises with 28S but not with 18S RNA (Fig. 2). Another fragment of 2.3 kb can be seen in the ethidium bromide stained pattern obtained with rDNA purified from a mixture of males and

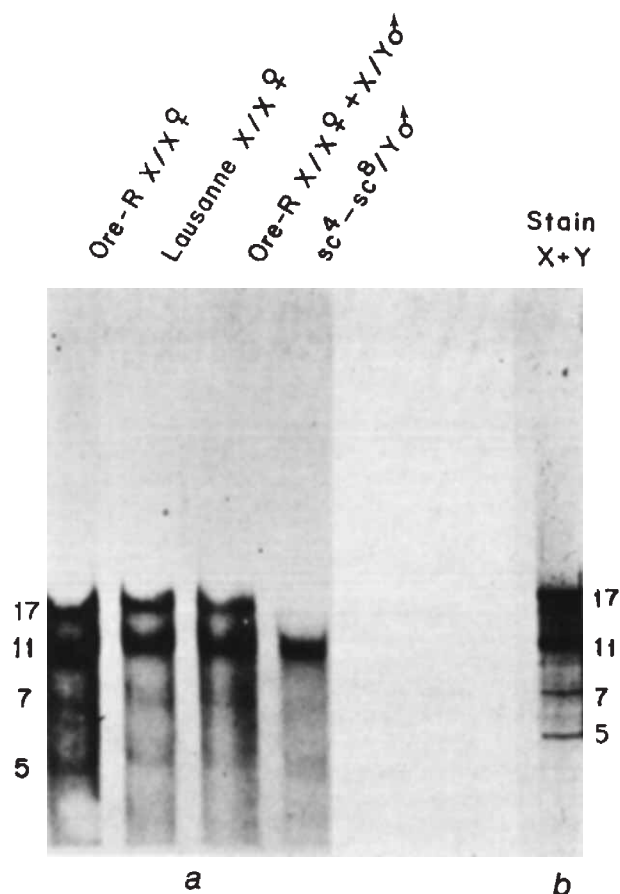


Fig. 1 *EcoRI* restriction pattern of *Drosophila* rDNA. The enzyme was prepared according to Greene *et al.*⁹. DNA of the indicated genotype was extracted, digested with *EcoRI* endonuclease and subjected to electrophoresis in 1% Agarose for 4 h at 100 V. The DNA fragments were transferred to a nitrocellulose filter⁷ which was then hybridised to ¹²⁵I-labelled rRNA, 5×10^7 c.p.m. μg^{-1} , for 24 h in $2 \times \text{SSC}$ at 60°C . The filter was treated with RNase ($30 \mu\text{g ml}^{-1}$) at 37°C for 60 min, dried and finally exposed to X-ray film for 5–10 d. The size of each fragment class is indicated in kb and was determined using *EcoRI*-treated λ DNA as the standard¹⁰. *a*, Ore-R wild-type female, Lausanne wild-type female, Ore-R female+male, and *sc*⁴-*sc*⁸/Y (Ore-R) male (*sc*⁴-*sc*⁸ designates an X chromosome that is deficient for its *NO*, see ref. 11); *b*, purified rDNA from a mixture of males and females that has been digested with *EcoRI* with the resulting fragments separated by electrophoresis and then stained with ethidium bromide.

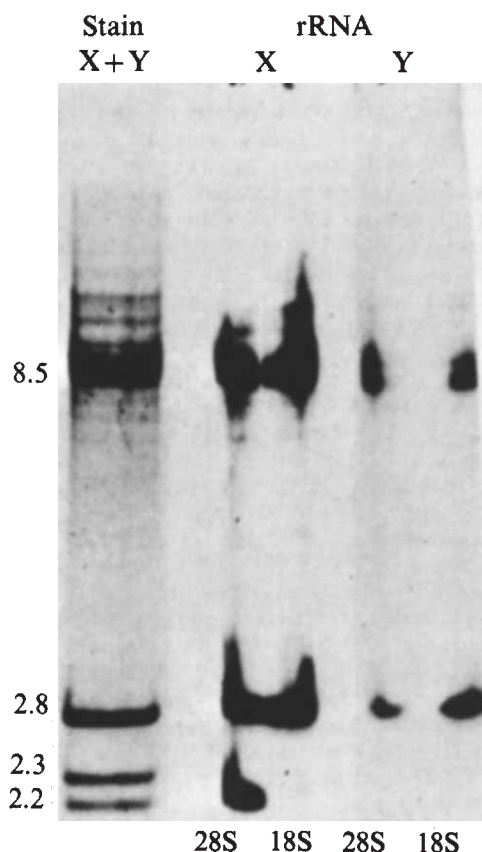
females, but it does not hybridise with rRNA and does not appear in the autoradiogram as do the other fragments. Thus the analysis with *SmaI* again confirms the presence of a structural difference between X and Y chromosome rDNA.

Fragment patterns of rDNA from *bobbed* mutants

To gain some insight into the relative interspersal of 17- and 11-kb units among each other we also analysed the fragment pattern obtained with *EcoRI*, using rDNA from four independently isolated X chromosome *bobbed* mutations. These mutations are deleted for 50–60% of their rDNA. If the *NO* consists principally of clusters of 17- and 11-kb units segregated from each other, then mutants deleted for 50% of their rDNA would be expected to be under represented for one class of the repeated units. Figure 2*b* shows that the rDNA in these mutants contains both the 17- and 11-kb size classes and that they are in roughly the same proportion to each other as in the wild type. Similar results have been obtained with six other independently

derived *bobbed* mutants. Because all these *bobbed* mutants arose by the deletion of half the rDNA repeats from an original *NO* and because each deletion arose independently, the results argue for interspersal of 11-kb and 17-kb repeat lengths. It is interesting that conspicuous length heterogeneity also occurs in the rDNA of most *bb* mutants (except *bb*st). The *bb*st mutant is actually a doublet in the 11-kb region. One band is 11 kb, the other 10 kb. *bb*st is extremely heterogeneous in the range 11 to 9 kb, revealing no discrete size class at the resolution of our gel, while *bb*⁴ contains bands at 11 and 9 kb and many smaller bands. Such extensive heterogeneity has been observed consistently among *bobbed* mutants. In wild-type rDNA length heterogeneity does exist, but most repeating units occur as the 11- and 17-kb size classes (Fig. 1*b*). One possible explanation for this heterogeneity is that *bobbed* mutants result in the preferential retention of some minor size classes during deletion of predominantly 11- and 17-kb repeats. The heterogeneity of lengths of *EcoRI* fragments in *bb*⁴, however, are so extensive that they cannot be explained in this way and we must consider additional mechanisms for generating such variations. Perhaps this length heterogeneity is a consequence of the event that initially generated the *bb*⁴ partial deletion.

Fig. 2 Fragments obtained from *Drosophila* rDNA with *SmaI*. The restriction enzyme was purified and used according to C. Mulder (personal communication). Fragments were separated and hybridised as described for Fig. 1. The stained pattern (ethidium bromide, $1 \mu\text{g ml}^{-1}$) was obtained from purified rDNA (X/X females and X/Y*bb* males; because the X contains about 200 rRNA genes and the Y*bb* about 120, 83% of the rDNA of this stock is derived from the X). The purification of the rDNA using gradient centrifugation in the presence of mercury and actinomycin D will be published elsewhere. The X and Y chromosome rDNA was partially purified before digestion, electrophoresis and hybridisation. Each filter lane was cut in half lengthwise and the left half was hybridised with 28S RNA, the right half with 18S RNA. The length of different fragments is given in kb.



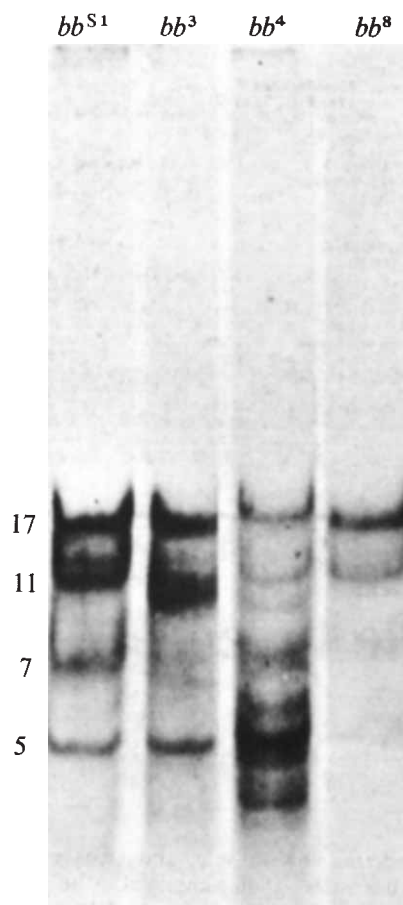


Fig. 3 *EcoRI* restriction pattern of rDNA from bobbed mutants. DNAs from females homozygous for bb^{S1} , bb^3 , bb^4 and bb^8 mutations were extracted, treated with *EcoRI* and analysed by electrophoresis and hybridisation to ^{125}I -rRNA as described in Fig. 1.

Genetic mechanisms in the evolution of rDNA

The results of the experiments described here, and summarised in Fig. 4, have interesting implications for the mechanism of redundant gene evolution. If the X and Y NOs were to recombine with each other (and these would necessarily have to be double exchanges to maintain intact X and Y chromosomes; Fig. 4), and different repeat lengths are interspersed, then both NOs would be expected to display similar *EcoRI* fragment patterns. The fact that the 17-kb fragment is present on the X but absent from the Y chromosome supports earlier genetic evidence against recombination between X and Y rDNAs. It then becomes necessary to explain the fact that both the X and Y NOs contain apparently identical 18S and 28S RNA sequences³, and share three of the four major discrete size classes of *EcoRI* fragments and at least one discrete fragment (2.8 kb) of the *SmaI* digest (Fig. 4). Unless genetic exchange between the X and Y NOs occurs on some occasions, we conclude that the similarities in the rDNA of these two NOs are maintained by selection pressure.

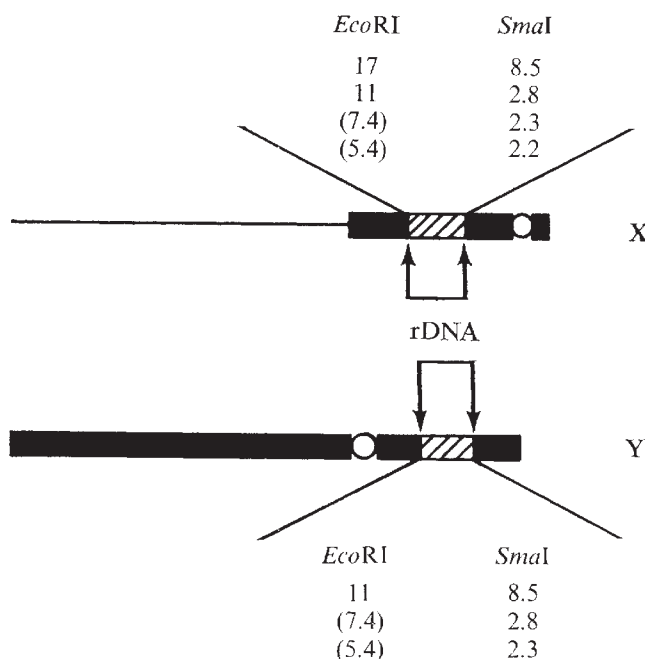
Drosophila is not unique in maintaining homologous genes on more than one chromosome. In humans, five chromosomes each contain a single NO^{14,15}. In various organisms the same satellite DNA sequence is frequently found to cluster at the centric heterochromatic regions on several non-homologous chromosomes¹⁶. In *Xenopus*, the 5S RNA genes are located at the telomeres of at least 15 chromosomes¹⁷. These are but a few examples which illustrate the variety of homologous genetic sites that have been found to

be dispersed throughout the genome of a given species. In the case of the *Xenopus* 5S genes, it has been suggested that the relative homogeneity among the 15 gene clusters has been maintained by recombination^{17,18}.

In the examples cited above it has not been tested whether the homologous genes or satellites found on different chromosomes are truly identical or differ in some structural aspects. Furthermore, although there is some cytological evidence for recombination between some of these homologous regions on non-homologous chromosomes, as in the case of *Xenopus* 5S DNA¹⁸, there is no direct genetic or physical evidence for such exchanges.

The situation with *Drosophila* rDNA poses a dilemma, in that two opposing interpretations of the available evidence may be proposed, both of which necessitate certain assumptions. The selection hypothesis is based on the prior genetic evidence against X-Y crossover events and places weight on the absence of 17-kb rDNA fragments on the Y NO. With these points in mind, we conclude that the X and Y NOs are indeed genetically isolated from each other. The apparent identity of the primary structure of the 18S and 28S rRNAs³, and the similarity of the restriction fragment patterns which we observe would then be due to selection pressures. Although it is difficult to understand how selection would be sensitive to details of the spacer structure and to every nucleotide in the rRNA sequence, it must be remembered that the functional significance of spacer DNA and all of the structural gene information is still obscure. On the other hand, the recombination hypothesis asserts that genetic exchanges in the form of double crossing-over events do take place between the X and Y NOs. Such exchanges would not be detected by genetic experiments, and they would account for those similarities between the X and Y rDNAs which exist. This interpretation, however, necessitates the assumption that rDNA repeats of the 17-kb class

Fig. 4 A summary of the similarities and differences in the structure of X and Y rRNA genes. Since one-third of the X DNA resides in the heterochromatic block¹³ (indicated as the darkened portion of the X) and since there are about 250 rRNA genes per X NO³, this means that the X rDNA accounts for about 30% of DNA in the heterochromatin of this chromosome. Similarly, about 30% of the DNA in the short arm of the Y is rDNA. *EcoRI* and *SmaI* fragment sizes are given in kb. Infrequent *EcoRI* size classes such as those seen in Fig. 1b have not been included. Parentheses around certain size classes indicate they are minor components.



either cannot participate in genetic exchange or are rapidly eliminated from the Y chromosome *NO*. This is a purely *ad hoc* assumption without any supporting experimental basis.

Our observations do not allow an unequivocal conclusion on the genetic and evolutionary mechanisms which determine the structure of *Drosophila* rDNA, and which may operate in clusters of repetitive genes in general. Nevertheless, this work points up difficulties with the hypothesis that genetic exchanges are the main mechanism which assures the similarity of homologous regions on non-homologous chromosomes, and raises the possibility that selection pressures play a significant role in the parallel evolution of families of repetitive genes.

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Significance of impulse activity in the transformation of skeletal muscle type

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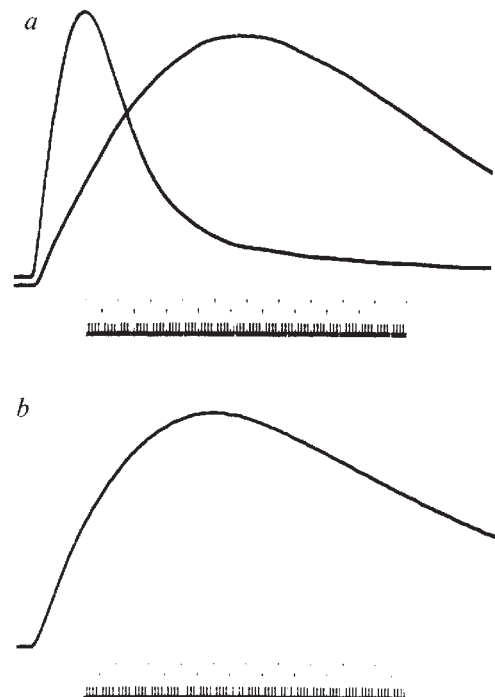
The changes which follow cross reinnervation of mammalian fast and slow twitch muscles may reflect a capacity of skeletal muscle to respond adaptively to different functional requirements. This interpretation is supported by experiments in which long-term electrical stimulation was used both to reproduce and to oppose the effects of cross reinnervation.

IN adult mammals the motoneurons innervating slow twitch skeletal muscles generate a sustained low-frequency pattern of activity; in contrast, motoneurons supplying fast twitch muscles subject them to intermittent bursts of more intense activity¹. The characteristic physiological and biochemical properties of the muscles emerge late in development, usually after birth, and in slow muscles this process seems to depend on the establishment of the adult pattern of motoneurone activity. If the motoneurons are rendered quiescent by isolation of the spinal cord a few days after birth, muscles which would normally have acquired slow contractile characteristics develop as fast muscles². Even in the adult animal a fast muscle subjected to low frequency activity by an implanted stimulator³ undergoes profound changes in all the properties which normally distinguish it from a slow muscle⁴⁻⁸. It has been pointed out^{4,9} that this capacity for change would enable a muscle to accommodate the changing functional requirements which may be encountered during an animal's lifetime. According to this view, the late phase of differentiation of slow muscles in the neonate and the changes resulting from chronic stimulation of fast muscles in the adult both represent an adaptive response to continuous, low-frequency activation. The question then arises as to whether such a concept can provide insight into previous demonstrations of plasticity of skeletal muscle properties. The most important of these are the cross reinnervation experiments pioneered by Buller *et al.*⁹.

In these experiments the motor nerves of a fast and a slow muscle were cut and cross anastomosed; as a result, the fast muscle was reinnervated by the nerve which pre-

viously supplied the slow muscle, and vice versa. The contractile characteristics then changed, the fast muscle becoming slower and the slow muscle faster. The authors considered, but were not convinced by, the possibility that these changes were due to changes in the patterns of impulse activity reaching the muscles. Instead they proposed that the contrasting properties of slow and fast muscles were

Fig. 1 Isometric twitch contractions of left and right EDL muscles (a) and right soleus muscle (b) recorded on identical time scales. The left EDL muscle (slower time course) has been stimulated at 10 Hz continuously for 20 weeks. Each time scale represents 100 ms with 1-ms divisions. Peak tensions are given in Table 1.



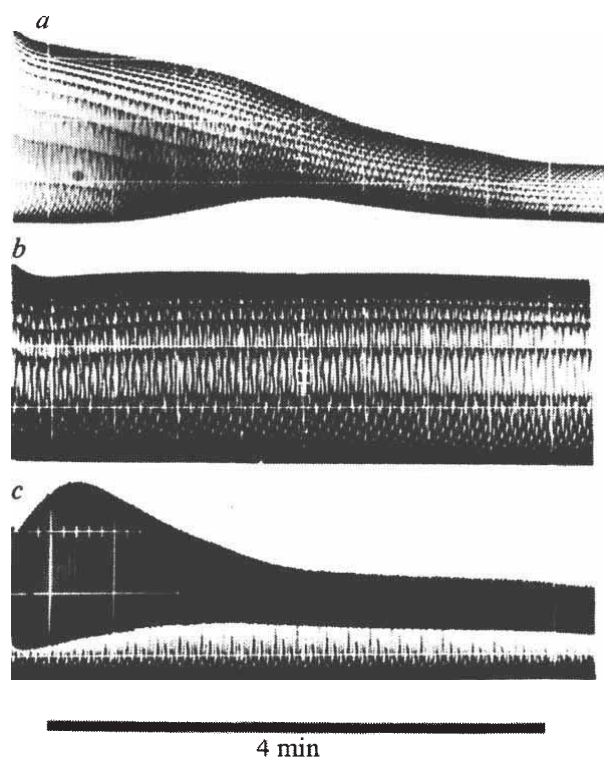


Fig. 2 Response of control soleus (*a*), stimulated EDL (*b*) and control EDL (*c*) muscles to a prolonged train of unfused tetani (25 Hz for 500 ms repeated every 1.25 s). The stimulated muscle is remarkably resistant to fatigue in these taxing conditions.

attributable to chemical trophic influences exerted by their respective motor nerves; cross union of the nerves caused the trophic substances to be carried to muscles of the opposite type, which were then transformed in accordance with the new innervation. Trophic concepts were not foreign to developmental biology, and the hypothesis was widely supported.

If cross union of the nerves interchanges the characteristic patterns of impulse activity reaching the slow and fast muscles, changes of the type observed would be in accordance with the adaptive-response hypothesis. The fast muscles, subjected to a continuous low frequency discharge, would tend to acquire slow muscle properties; the slow muscles, relieved of such a pattern of activation, would tend to undergo reciprocal changes. Although it remains to be confirmed that the firing pattern of a given moto-

neurone pool is fundamentally the same after cross reinnervation as before, Yellin¹⁰ has reviewed indirect evidence which strongly supports this expectation.

Assuming, therefore, that there is a transposition of activity, can this alone account for the effects of cross reinnervation? This question has received balanced discussion (for example, refs 11 and 12) but has remained unanswered because of a lack of suitable data. We wish to report new evidence which makes it possible to compare, both qualitatively and quantitatively, the effects of cross reinnervation with those of a change in the pattern of impulse activity.

Long term stimulation

In their original work Salmons and Vrbová demonstrated marked slowing in response to long term stimulation in fast muscles of the rabbit and cat⁵. The results were confirmed and extended by two other groups, using different techniques of stimulation^{13,14}. These experiments showed that the effects of stimulation were significant and were not confined to a particular muscle or species, but the duration of stimulation was not sufficient to afford a basis for comparison with cross reinnervation experiments. As a result of improvements in the design of the stimulator and in the surgical technique for its implantation, we have been able to stimulate fast muscles of the rabbit continuously for much longer periods than before. We present here the results of two experiments, each of 20 weeks' duration.

The animals were operated on in strictly aseptic conditions. In each case the stimulator capsule was implanted intraperitoneally, and the two flexible leads were passed subcutaneously to the left hind limb and fixed near the common peroneal nerve. The right limb served as an unoperated control. The animals were returned to the colony for the ensuing period, during which they remained in excellent condition and showed a normal gain in body weight. After 20 weeks a terminal procedure was carried out in which isometric twitch, tetanus and fatigue characteristics were determined for the tibialis anterior (TA), extensor digitorum longus (EDL) and soleus muscles of both hind limbs. Each muscle was set at the resting length at which it produced the maximum twitch contraction. Recordings from corresponding muscles of the two sides were made simultaneously in controlled thermal conditions. The muscles were then quickly excised, weighed, frozen in liquid nitrogen, sealed hermetically in polyethylene sachets and stored in solid CO₂ for biochemical analysis.

Figure 1 compares the twitch contraction of a stimulated EDL muscle with that of control EDL and soleus muscles of the contralateral limb. It is evident that the stimulated EDL muscle contracts even more slowly than a classical

Table 1 Effects of long term stimulation on a fast muscle

	Control TA	Stimulated TA	Control soleus
Maximum twitch tension (g)	370	455	251
Time to peak twitch contraction (ms)	17.0	64.9	57.2
Time from peak to half-relaxation (ms)	17.9	77.1	91.1
Maximum tetanic tension P_0 (g)	3919	1829	1254
Twitch: tetanus ratio	0.094	0.249	0.200
Maximum rate of rise of tetanic tension ($\%P_0 \text{ ms}^{-1}$)	3.06	1.11	1.73
Ca ²⁺ -activated myosin ATPase activity ($\mu\text{mol mg}^{-1} \text{ min}^{-1}$)	0.66	0.18	0.22
Ca ²⁺ -activated myosin ATPase activity after preincubation at pH 9.2 ($\mu\text{mol mg}^{-1} \text{ min}^{-1}$)	0.68	0.06	0.06
Ca ²⁺ uptake by FSR; initial rate ($\mu\text{mol mg}^{-1} \text{ min}^{-1}$)	2.19	0.21	0.56
Total uptake in 15 min ($\mu\text{mol mg}^{-1}$)	3.81	0.55	1.52

Physiological and biochemical characteristics of the muscles whose twitch contractions are illustrated in Fig. 1. Fragmented sarcoplasmic reticulum (FSR) and myosin were prepared as reported earlier^{20,21}. To determine the alkali lability myosin was preincubated at pH 9.2 in a solution containing 0.2 mg of protein per ml, 25 mM KCl, 10 mM Tris, 10 mM CaCl₂ for 10 min at 25 °C. At the end of this preincubation period the pH was readjusted to 7.6 by adding Tris to a final concentration of 50 mM and HCl as required. The ATPase reaction was then started by adding ATP.

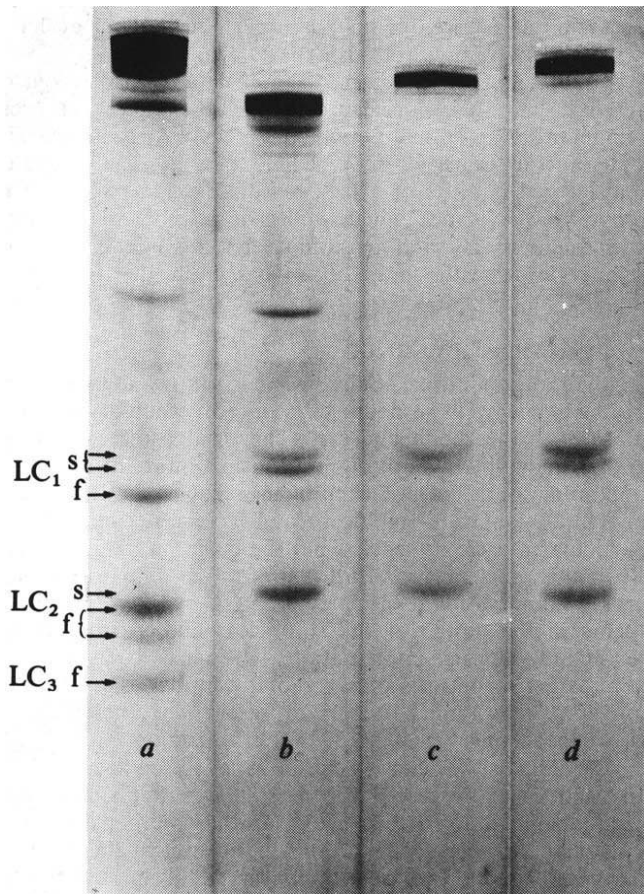


Fig. 3 SDS-polyacrylamide gel electrophoretograms of myosin prepared from control TA muscle (*a*) TA muscle stimulated for 20 weeks (*b*), EDL muscle stimulated for 20 weeks (*c*), control soleus muscle (*d*). Myosin light chains characteristic of slow muscle are demonstrable after 4 weeks of stimulation¹⁵. Continued stimulation results in the successive disappearance of the fast muscle light chains, in the order LC_{2f} , LC_{3f} , LC_{1f} (refs 6 and 8).

slow muscle in the same animal. Similar results were obtained for the other stimulated fast muscles. In response to a prolonged train of 25-Hz tetani, the stimulated muscles proved to be even more resistant to fatigue than the solei, and showed, in the early phase of the fatigue record, the slight post-tetanic depression characteristic of slow muscles in place of the marked potentiation normally seen in fast muscles (Fig. 2). The biochemical results were no less striking, and showed a continuation of the trend described previously⁶. Table 1 illustrates typical data from one muscle pair. In addition to changes in these parameters, electrophoretograms of myosin light chains on sodium dodecyl sulphate (SDS)-polyacrylamide gels revealed that the light chains characteristic of fast muscles had been replaced entirely by light chains typical of slow muscles (Fig. 3). There was also evidence that the heavy chains of myosin had been replaced, since myosin from the stimulated muscle lacked the *N*- τ -methylhistidine amino acid residue characteristic of fast muscle heavy chains. Both these observations agreed with our earlier findings for shorter periods of stimulation^{6,8,15,16}.

These results represent a degree of transformation which matches or exceeds that of muscles cross reinnervated for much longer periods. We have shown, for example, that stimulation even for 12 weeks is enough to bring about the replacement of light and heavy chains of myosin noted above, yet both processes are still incomplete 1 yr after cross reinnervation^{8,18}. The effects of a change in the pattern of impulse activity are therefore sufficient to account both

qualitatively and quantitatively for the changes which result from cross reinnervation.

Equality of effect, however, does not necessarily imply equivalence of mechanism. It could be argued that changes in impulse activity and cross union of motor nerves achieve similar end results by different routes. To examine this possibility, we designed a null experiment in which the effects of long term stimulation would be set in opposition to those of cross reinnervation (for a preliminary account see ref. 17).

Stimulation versus cross reinnervation

In a group of 20 rabbits the common peroneal nerve and the motor nerve to the soleus muscle were exposed using a lateral approach (A. R. Luff, personal communication). One of the branches of the deep peroneal nerve supplying motor fibres to the fast muscles TA and EDL was carefully dissected free, cut and gently apposed to the peripheral cut end of the soleus nerve, using a single suture of 9/0 or 10/0 nylon. The reciprocal cross union was not performed, to provide conditions which favoured cross reinnervation of soleus but militated against self reinnervation by its original nerve. Since the bulk of the common peroneal nerve was left intact, the functional impairment incurred by this operation was minimal. Eight weeks after cross union, a stimulator was implanted in each of ten animals, subjecting the

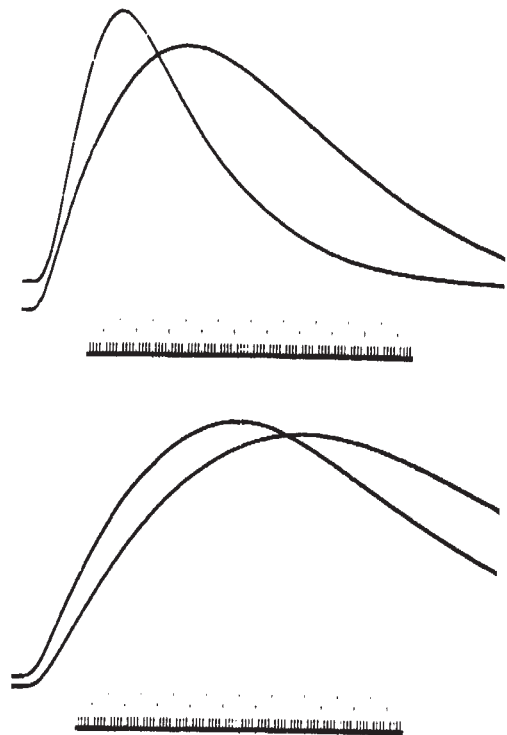


Fig. 4 Isometric twitch contractions of soleus muscles. In each case the left muscle of the pair has been reinnervated by the motor nerve from a fast muscle as a result of nerve cross union 16 weeks previously. In the upper picture the left muscle shows the increase in contractile speed which is the well documented consequence of cross reinnervation. The left muscle in the lower picture had been subjected to tonic stimulation through the new innervation for the previous 8 weeks; it exhibits a time course of contraction slightly more prolonged than that of the control muscle. Contractions of the left soleus muscles were elicited by stimulation of the left common peroneal nerve. In this and all other experiments of the series evidence for self reinnervation was sought by stimulating the left tibial nerve. The tension record then showed a small excursion of irregular shape and rapid time course (time to peak 20.07 ± 2.30 ms) which we attribute to residual mechanical cross-talk from the subjacent flexor hallucis longus muscle. In no instance was there any evidence of the slow twitch contraction which would have been indicative of self reinnervation.

Table 2 Characteristics of cross-reinnervated soleus muscles subjected to stimulation

	(a) Cross innervation alone		(b) Controls		(c) Cross innervation & stimulation
Time to peak twitch contraction (ms)	25.7±0.7 (n=6)	(P < 0.001)	55.5±2.9 (n=16)	(P > 0.2)	63.5±8.3 (n=6)
Time to half-relaxation (ms)	26.7±1.6 (n=6)	(P < 0.001)	74.2±5.0 (n=16)	(P > 0.9)	72.9±13.7 (n=6)
Twitch-tetanus ratio	0.15±0.02 (n=5)	(P < 0.01*)	0.21±0.01 (n=15)	(P > 0.2*)	0.16±0.03 (n=4)
Max. rate of rise (%P ₀ ms ⁻¹)	2.86±0.17 (n=5)	(P < 0.001)	1.74±0.14 (n=13)	(P > 0.2)	1.36±0.21 (n=4)
Ca ²⁺ -activated ATPase (μmol mg ⁻¹ min ⁻¹)	0.48±0.06 (n=6)	(P < 0.001)	0.26±0.02 (n=27)	(P > 0.6)	0.28±0.04 (n=5)
Alkali lability (% loss ATPase activity)	12.4±6.3 (n=6)	(P < 0.001)	60.6±2.8 (n=15)	(P > 0.5)	57.7±2.6 (n=5)
Ca ²⁺ uptake by FSR: initial rate (μmol mg ⁻¹ min ⁻¹)	1.42±0.24 (n=6)	(P < 0.01)	0.64±0.11 (n=17)	(P > 0.2)	0.36±0.09 (n=5)
total uptake (μmol mg ⁻¹ per 15 min)	2.19±0.10 (n=6)	(P < 0.001)	1.33±0.09 (n=17)	(P > 0.8)	1.15±0.31 (n=5)
ATPase (μmol mg ⁻¹ min ⁻¹)	1.68±0.29 (n=6)	(P < 0.05)	1.14±0.12 (n=17)	(P > 0.2)	0.81±0.17 (n=5)

*Paired *t* test.

Physiological and biochemical characteristics (mean±s.e.m.) are given for: *a*, soleus muscles cross reinnervated by a fast muscle nerve; *c*, muscles treated similarly but subjected to chronic stimulation during the last 8 weeks of the 16-week experimental period, and *b*, control soleus muscles. The data in columns (*a*) and (*c*) have been compared with control data in column (*b*) and *P* values for the differences assigned in each case. Except where noted, significance was assessed using Student's *t* test on the unpaired data. Additional control muscles have been included to establish reliable baselines and to provide more stringent conditions for the test of non-significance on the right hand side. In spite of the low mean value for the twitch-tetanus ratio in column (*c*), two of the animals in this group showed a higher twitch-tetanus ratio for the experimental muscle than for the contralateral control muscle. Of the muscles subjected to cross reinnervation alone (column (*a*)), none had a twitch-tetanus ratio greater than 60% of that of the contralateral control muscle.

cross reinnervated soleus muscle to a continuous train of impulses at 10 Hz delivered through the new innervation. These muscles would therefore have a pattern of activation substantially similar to that which preceded cross innervation. In the remaining animals dummy electrodes were implanted which were not connected to a stimulator. The rabbits were operated in matched pairs so that the two groups comprised animals with comparable histories. After a further 8 weeks the muscles were examined in a terminal procedure similar to that described above.

The adaptive response hypothesis required that the usual consequences of cross innervation be totally absent in the muscles which received stimulation. This expectation was indeed borne out. In Fig. 4 twitch contractions of soleus muscles from one pair of animals are compared. In the animal with dummy electrodes the cross innervated soleus muscle shows the usual substantial increase in the speed of contraction and relaxation. In the animal with a stimulator the cross innervated and stimulated soleus muscle contracts and relaxes even more slowly than its counterpart on the control side. Table 2 gives values for a broad range of physiological and biochemical variables measured in the two groups of animals. In each parameter the cross reinnervated soleus muscles differed significantly from the control muscles, whereas the muscles which were both cross reinnervated and stimulated did not differ significantly from the controls. Gel electrophoretograms of myosin light chains confirmed the presence, in cross reinnervated soleus muscles, of light chains characteristic of fast muscle, as reported previously¹⁸; on the other hand, cross reinnervated soleus muscles. Since it is highly unlikely that different chain patterns indistinguishable from those of control soleus muscles. Since it is highly unlikely that different mechanisms could produce such a detailed complementarity of effect we conclude that the effects of impulse activity and those of cross reinnervation are mediated in the same way.

A converse experiment would be to cross reinnervate a fast muscle with a nerve which previously supplied a slow muscle, and then relieved the muscle of the activity imposed

by the new innervation. In fact this experiment formed part of the original study by Buller *et al.*⁹. No effects attributable to cross reinnervation could be discerned when steps had been taken to abolish normal motor activity by section of the spinal cord and lumbosacral dorsal roots. It was noted at the time that this observation could be interpreted within the framework of the trophic hypothesis if the synthesis, transport or release of trophic substances were dependent on the passage of nervous impulses. The finding is, however, equally well understood in terms of a more direct role of impulse activity, and as such is fully consistent with the other results presented here.

In summary, the data now available point to two general conclusions. (1) In the absence of an actual change in the pattern of impulse activity reaching a muscle, cross reinnervation has no significant effect. (2) A change in the pattern of impulse activity unaccompanied by a change in the motor innervation can produce effects equalling or surpassing those brought about by nerve cross union.

Implications for nature of neural influence

These observations rule out the possibility that the physiological and biochemical differences between fast and slow muscles are attributable to fundamental differences in the chemotrophic character of their motor nerves. Therefore in seeking an explanation of the neural influence on muscle differentiation we may confine our attention to impulse-dependent mechanisms. The data are not incompatible with the secretion of a trophic substance or substances by those α motoneurons, irrespective of type, which sustain a particular pattern of activity¹⁹. Such an hypothesis, however, now seems unnecessarily elaborate, for we have shown that the same phenomena can be explained just as adequately in terms of events taking place wholly within the muscle. According to this interpretation, gene expression in skeletal muscle is subject to the regulatory influence of one or more substances whose intracellular concentrations alter as a consequence of changes in the level of contractile or metabolic activity. Such a shift in emphasis from explicit chemical control by the central nervous

system to adaptive regulation by the muscle may prove to be a more fruitful concept in the design of future experiments in this area.

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letters to nature

Spectral characteristics of transient X-ray sources

THE transient X-ray sources A1524–62 and A0535+26 were briefly observed by the University College, London, proportional counter spectrometer, experiment C on the Ariel V satellite (P. W. Sanford and J. C. Ives, unpublished). The resulting spectra help to complete the spectral history of these two sources, making seven sources for which there is now extensive spectral information. A general scheme emerges from these in which the sources with hard spectra ($n \lesssim 2$) show little spectral evolution and those with soft spectra ($n > 2$) exhibit marked softening with time. We discuss here this subdivision in relation to other properties of the sources.

The X-ray transient A1524–62 was first reported by Pounds¹ and a detailed light curve has been presented by Kaluzienski *et al.*² These authors reported that the spectrum before peak intensity showed a slight softening, with the data at the precursor maximum being well fitted by a power law spectrum of photon index -2.5 and with no measurable soft X-ray absorption ($< 10^{22}$ atoms cm^{-2} , 1σ). Their data were not consistent with an isothermal Bremsstrahlung X-ray spectrum.

This source was observed by experiment C for two days, February 6–8, 1975, well after peak intensity, and the mean intensity in the 3–6-keV energy band was found to be 0.183 ± 0.001 photons $\text{cm}^{-2} \text{s}^{-1}$ (1σ), in good agreement with Kaluzienski *et al.*² The orbit-by-orbit intensities showed no variability

outside the 3σ uncertainties, setting an upper limit of 10% on intensity changes at the time scale of hours.

The spectrum obtained by integrating the data over this period is shown in Fig. 1 together with the best fitting power law spectrum. The best fitting parameters for both power law and exponential trial spectra are given in Table 1. The power law spectrum (index 4.23 ± 0.07) gives a better fit to the data,

Fig. 1 The spectrum obtained during the 2-d observation of A1524–62. The continuous curve is the best fitting power law spectrum whose parameters are listed in Table 1.

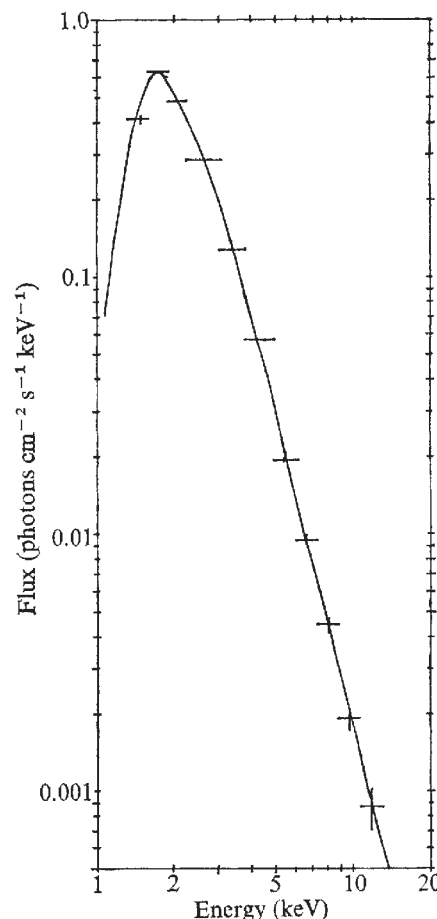


Table 1 Best fitting spectral parameters for A1524–62 and A0535+26

	Power law spectrum $dN/dE = AE^{-n} \exp(-N_H\sigma)$	Exponential spectrum $dN/dE = A \exp(-E/kT) E^{-1} \exp(-N_H\sigma)$
A1524–62		
A0535+26		
A	30.6 ± 3.0	5.2 ± 0.5
n or kT	4.23 ± 0.07	1.43 ± 0.05
N_H	$(3.40 \pm 0.07) \times 10^{22}$	$(7.1 \pm 0.8) \times 10^{21}$
χ^2 for 8 d.f.	256	465
A	1.3 ± 0.08	Data not
n or kT	0.67 ± 0.03	consistent with
N_H	No detectable cutoff ($< 1 \times 10^{22}$)	single temperature
χ^2 for 29 d.f.	14	fit

σ is the Brown and Gould³ absorption cross section. The errors are 1 s.e., and are calculated by the least squares program used to fit the trial spectra.

Table 2 Summary of the characteristics of the seven most extensively observed transient X-ray sources

Source	τ_r (d)	τ_1 (d)	τ_2 (d)	Rise	Spectral parameters*		Decay 1	Decay 2	$I_{\max}$ ($\text{erg cm}^{-2} \text{s}^{-1}$)	D (kpc)	$L_{\max}$ (erg s^{-1})	Comments (OC = Optical candidate)
Cen X-4 refs 7, 8	4	6	40	n	1	2	3	3	5×10^{-7}			
3U1543-47 refs 9, 10	4	10	100	n	3	4	4	4	4×10^{-8}	3	4×10^{37}	OC = Variable (ΔM = 0.06) M giant
A1524-62 see text	4	30	60	N_H	$1.5-2.5$	$< 10^{22}$		6×10^{21}	5×10^{-9}			
A1118-61 refs 11-13	3	7		n	$1.0^\dagger$	$0.9^\dagger$	$1.1^\dagger$	3.4×10^{22}	4×10^{-9}	3	4×10^{36}	Pulsating ($P = 405$ s). OC = Variable (ΔM = 0.1) Be Star
A1743-28 refs 14, 15		18	90	n			3	3	3×10^{-8}	$10^\dagger$	3×10^{38}	
A0535+26 refs 16, 17	6	20		N_H	0.67	0.8	1.1	10^{23}	5×10^{-8}	1	5×10^{36}	Pulsating ($P = 104$ s). OC = Variable (ΔM = 0.7) Be Star
A0620-00 refs 18, 19	5			n	1.6	5	5		1×10^{-8}	2	4×10^{18}	OC = Blue star, var- ied by $\Delta M \approx 8$ in co- incidence with X-ray outburst
				N_H	$< 10^{22}$	5×10^{22}	3.9×10^{21}	(1.3 keV)				

τ_r = Time for intensity to increase from ($I_{\max} e^{-1}$) to $I_{\max}$; τ_1 = time for intensity to decrease by a factor e after maximum intensity; τ_2 = sources Cen X-4, 3U1543-47, A1524-62, A1742-28 exhibited a second slower decay of e -folding time τ_2 . Sources Cen X-4, 3U1543-47 showed a further rapid decay, after $(1-2) \times \tau_2$, leading to their disappearance: $I_{\max}$ = intensity at maximum; D = estimated distance; $L_{\max}$ = luminosity at maximum.

*For comparison, all spectra are described in terms of photon indices. Where not quoted directly, it has been roughly calculated from the reported temperature, which is also quoted in parentheses.

† Unpublished revised values of n and N_H , following calibration of Ariel V experiment C on the Crab Nebula.

‡ Distances have been estimated from proposed optical identifications, except for A1742-28, the galactic centre nova, for which the 10 kpc is based on the celestial position and observed high column density².

but both spectra require significant soft X-ray absorption, equivalent, in the case of the power law fit, to a neutral hydrogen column of $(3.40 \pm 0.07) \times 10^{22}$ atoms cm^{-2} . The spectrum thus shows that a considerable spectral softening occurred in the source at or after maximum intensity.

The transient A0535+26 was observed by one or more of the Ariel V satellite experiments during the period April 13-June 6, 1975, and again between June 21 and August 15, 1975. The light curve (A. P. Willmore, unpublished) is fairly typical of other transient X-ray sources, with a precursor peak, a rise to maximum intensity (on May 1), followed by a quasi-exponential decay (time constant ~ 20 d). A subsidiary peak of 15% of the maximum has also been reported.

A0535+26 was observed by experiment C on April 28, that is, before maximum. The resultant data are shown in Fig. 2, together with the higher energy data from the crystal scintillator experiment on Ariel V (ref. 4). The experiment C data are best fitted by a power law spectrum with photon index of 0.67 ± 0.03 , without measurable cutoff (see Table 1). However, neither a power law spectrum nor a single temperature exponential spectrum is consistent with the combined low and high energy data. Ricker *et al.*⁵, from a balloon experiment on June 1, also found spectral steepening at higher energies not consistent with a single temperature exponential spectrum.

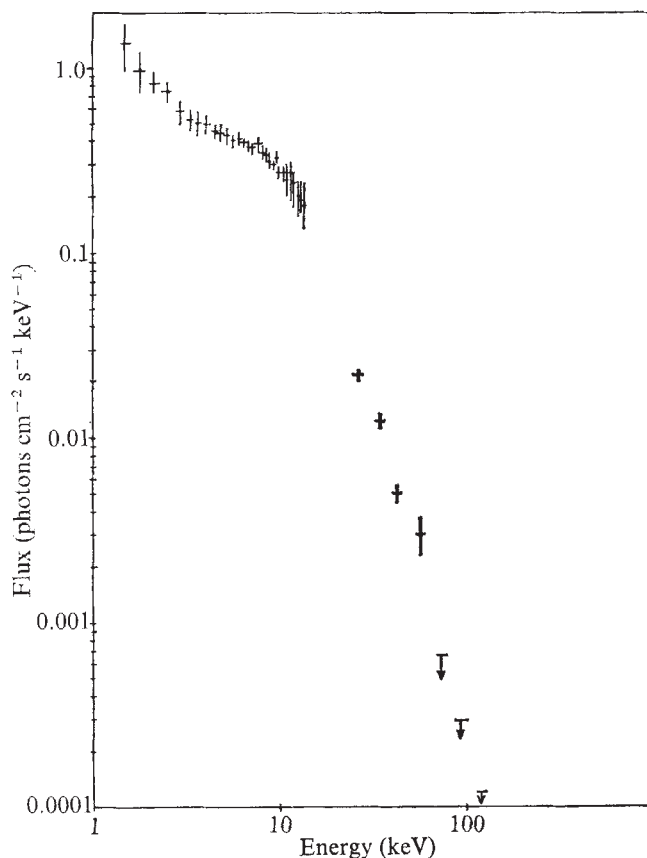
Comparison of the spectral data between 2 and 20 keV obtained by experiment C just before maximum, and by Ricketts *et al.*⁶, suggests a slight spectral softening during the decay at these energies, but not so marked as for other X-ray transients.

The information on the most extensively observed transients is summarised in Table 2. A detailed review concerning the 5 most recent sources has been given by Willmore (unpublished). We stress that the values reported in Table 2 not directly given in the quoted literature, but estimated on the basis of the available information, are only indicative. This applies to the majority of the quoted time scales and to some of the spectral indices.

All the time scales are broadly similar, ranging from a few weeks to a few months, but this probably arises from observational selection effects. In fact there are indications of both faster²⁰ and slower²¹ transients. Nonetheless, we are probably discussing a group of substantially similar events. Among the theories proposed for 'X-ray novae', variable accretion on to

compact objects in binary systems (refs 10, 15, 20, 22, 23, and F. K. Li, G. F. Sprott and G. W. Clark, unpublished) seems the most natural, in view of the similarities with known binary X-ray sources and the independent evidence of intermittent mass exchange in binary systems. The time scales should then be associated with the mechanism that drives the mass transfer.

Fig. 2 The spectrum of A0535+26. The experiment C data points for day 118, 1975 (1.5-15 keV) are combined with those reported by Coe *et al.*⁴ (20-120 keV) for days 116-118, 1975.



It is apparent from Table 2 that, in their spectral characteristics, A1118-61 and A0535+26 are similar to each other and different from the other five sources. The first two have very hard spectra ($n \approx 1$) which do not show strong variation with phase. The other five have spectral indices at, or after, maximum of $n \approx 3-5$ and in four cases where spectral observations before maximum are available, there is evidence that the spectrum has varied, evolving from a hard to a soft state. In three cases the variation is from $n \approx 1$ to $n \approx 3-5$.

Moreover, the two hard sources exhibit regular pulsation and the proposed optical counterparts are Be Stars ($M \gtrsim 10 M_{\odot}$), while for the other five pulsation has not been observed and the available optical identifications (for 3U1543-47 (ref. 24) and A0621-00 (ref. 25)) indicate low mass companions. (The absence of further possible identifications in this group is consistent with this.)

It has been pointed out (L. Maraschi, A. Treves and E. P. J. van den Heuvel, unpublished) that among all galactic X-ray sources there exists a strong correlation between the occurrence of pulsation, the hardness of the X-ray spectrum and the association with systems containing bright early type stars. The analogous grouping within the more limited sample of transients therefore acquires significance, and can be interpreted simply as reflecting the difference between the two classes of systems, of which Cen X-3 and Sco X-1 can be taken as prototypes. It is not clear why the spectral type of the primary star should be related to the properties of the X-ray emission from the compact companion and a possible model is discussed by Maraschi *et al.* The different spectral evolution of transients of the two groups is an important fact to be considered in models accounting for the different X-ray characteristics of the two types of binaries.

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The nature of association of equatorial spread F with magnetic activity

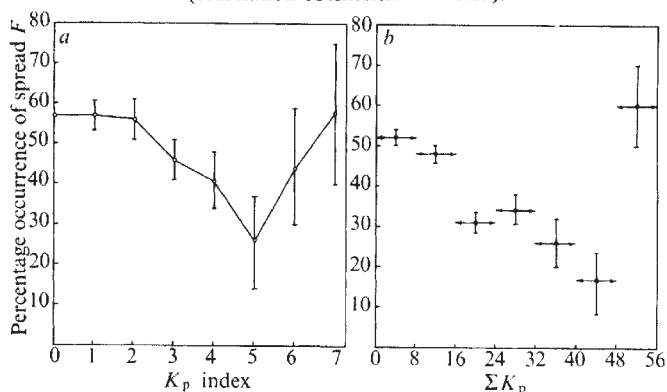
STATISTICAL studies based on a published data of f_oF_2 have revealed that equatorial spread F depends inversely on magnetic activity during the sunspot maximum period but tends to disappear or even become positive in some seasons during the sunspot minimum period¹⁻³. Somayajulu *et al.*⁴, using a different technique, considered the occurrence of spread F during different phases of magnetic storms, and they concluded that the initial storm phase does not have any inhibiting effect on spread F occurrence and that spread F occurs in general during the main phase of the magnetic storms. They observed that, during the recovery phase when the field value is quite different from the normal value, there is no spread F but it is present when the magnetic field value is close to its normal value. To investigate this discrepancy with the earlier results further, the spread F behaviour has been studied⁴ in relation to K_p indices, and for the Trivandrum data of 1970, and though the overall correlation between spread F and magnetic activity is negative, occurrence of spread F was found to decrease initially with increasing ΣK_p and then to start increasing at higher ΣK_p values. The effect of magnetic activity on equatorial spread F is reported here.

Equatorial spread F starts generally around 1900 LMT and lasts for a few hours. On some occasions, however, it persists throughout the night⁵. We have examined quarter hourly ionograms, during onset and development phases of spread F , from Trivandrum (dip -0.6° S) for 1970, and from Kodaikanal (dip -3.5° N) for 1969 and 1964, and the f -plots of Huancayo (dip -1° N) for 1968 to study the characteristics of spread F during its onset and development phase with magnetic activity as indicated by K_p indices. For this, the K_p indices for 1800-2100 LMT of Trivandrum and Kodaikanal and 1900-2200 LMT of Huancayo were used. The shift of 1h for Huancayo was used because K_p as given in the Solar Geophysical Data corresponds to that particular 3-h interval. The daily sum of K_p indices, represented by ΣK_p , was also calculated. The spread F data were grouped according to K_p and ΣK_p and percentage spread F was estimated for each (Figs 1-3).

In Figs 1-3, vertical bars indicate the 95% confidence limits. The yearly average sunspot numbers for the year 1968, 1969 and 1970 are 106, 106 and 105 respectively. These three years correspond to the period around sunspot maximum in that particular solar cycle and the year 1964 corresponds to the sunspot minimum (with a sunspot number of 10). In the following discussion no distinction is made between 1968, 1969 and 1970 in regard to the sunspot activity.

The most striking features of Figs 1, 2 and 3 is the reversal of the trend of variation of occurrence of spread F with increasing K_p value. This starts decreasing with increasing K_p but at a particular value of K_p it starts increasing again. It is

Fig. 1 Percentage occurrence of spread F at Trivandrum (75° E) against (a) the K_p index during 1800-2100 LMT (correlation coefficient = -0.19) and (b) against ΣK_p for the whole day (correlation coefficient = -0.29).



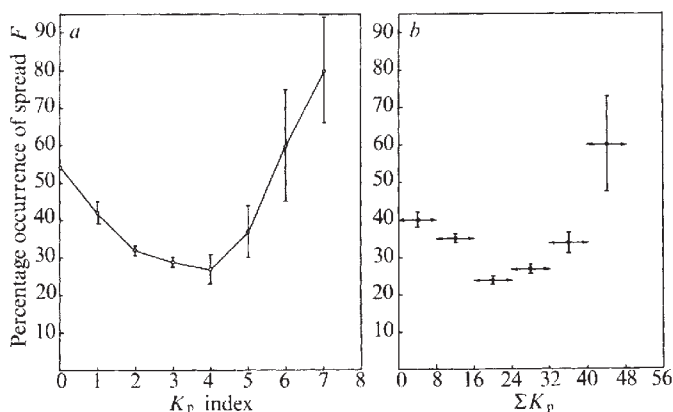
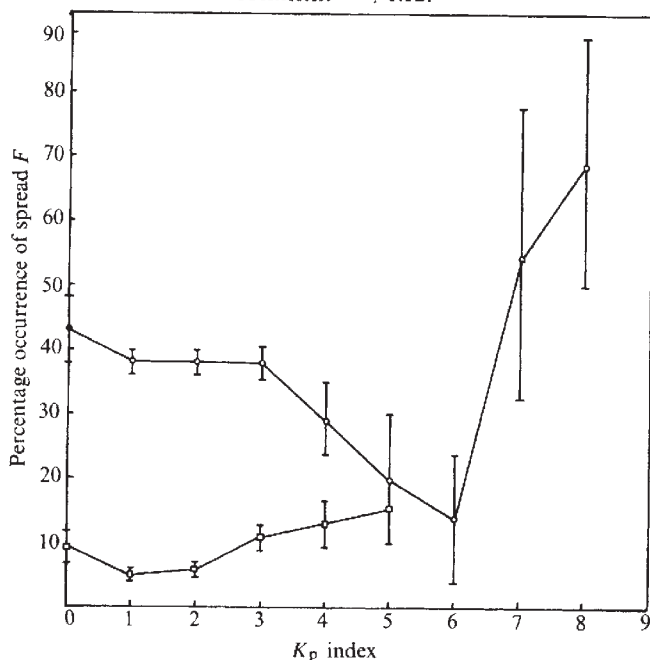


Fig. 2 Percentage occurrence of spread F at Huancayo (75°W) against (a) K_p index during 1900–2200 LMT (correlation coefficient = -0.12) and (b) ΣK_p during the whole day (correlation coefficient = -0.1).

even greater at the highest K_p value compared to that at the quietest condition ($K_p = 0$), as can be seen clearly from Figs 2 and 3. In Fig. 1 the two are almost equal. The value of K_p at which the reversal occurs at Huancayo is less than that at Trivandrum and Kodaikanal. Another important feature is that the decrease in spread F with K_p is rather slow initially at Trivandrum and Kodaikanal compared to that at Huancayo but later the opposite is true. For 1964 the general level of spread F is low at Kodaikanal as can be seen from Fig. 3. The reversal of the trend is seen as for the sunspot maximum year but with a major difference: it occurs at a much lower value of K_p ($= 1$). The correlation coefficients between spread F and K_p index are also shown in Figs 1, 2 and 3. They are all negative except for 1964 at Kodaikanal. The coefficients are all $> 95\%$ confidence limit. This shows that though the relationship between K_p and spread F reverses at high K_p , the overall correlation between the two remains negative in accord with the earlier results^{1–3}. The overall negative correlation obviously arises because days with high K_p value (or ΣK_p) are less numerous than those with low K_p value (or ΣK_p).

It is known that the onset of spread F is earlier in the period of sunspot maximum compared with its onset in sunspot minimum period. We have here considered the same 3-h

Fig. 3 Percentage occurrence of spread F at Kodaikanal (75°E) against the K_p index during 1800–2100 LMT. $\circ$, Data for 1969, correlation coefficient = -0.09 ; $\square$, data for 1964, correlation coefficient = $+0.12$.



period (1800–2100 LMT) both for sunspot maximum and minimum years for Kodaikanal. As such, the occurrence of spread F during the onset and development phase of spread F in the sunspot minimum period may be underestimated. Its variation with K_p may not, however, be affected.

The reversal at a lower K_p value for Huancayo during the sunspot maximum years is quite likely to be true for the sunspot minimum period also. One would then expect that the occurrence of spread F starts increasing right from $K_p = 0$. This may result in comparatively more spread F at Huancayo during sunspot minimum relative to that at sunspot maximum because there would be more days with low K_p than with high. In fact many investigators³ reported just this.

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v.l.f. emission from ring-current electrons

v.l.f. emissions associated with the enhancement of ring-current electrons during magnetic storms and substorms have been detected by the satellite S³-A (Explorer 45) on an equatorial orbit^{1,2}. The emissions observed near the geomagnetic equator consist of essentially two frequency regimes; one above the electron gyrofrequency at the equator, f_H —the electrostatic mode^{3–5}, which peak near $(n + \frac{1}{2})f_H$, where n is positive integer (emissions up to $n = 10$ have been observed²)—and the other below f_H —the whistler mode, which has a conspicuous gap at exactly $f_H/2$. Here we describe the characteristics of this v.l.f. emission and the associated enhancements in electron intensity as well as the anisotropies of the ring-current electron distribution, and we also give an interpretation of the bimodal frequency distribution of the equatorial whistler mode emissions. This distribution has been known for the past few years but has not been well explained^{6,7}.

Figure 1 is a part of the wide-band data obtained during the main phase of the December 17, 1971 magnetic storm¹. Figure 1a is the a.c. magnetic field data measured by the search-coil magnetometer with the upper cutoff near 3 kHz (ref. 8) and Fig. 1b is the a.c. electric field data obtained by the electric field sensor with the upper cutoff of 10 kHz (ref. 9). The figure (a and b) shows the time sequence of the observed emissions along the inbound orbit (No.101) of the satellite as f_H changes from ~ 3.0 kHz at 2000 UT to 6.0 kHz at 2100 UT. Although the automatic gain control of the sensor produces occasional spurious intensity variations, it should be noticed that emissions at frequencies $> f_H/2$ are generally stronger and more continuous than those $< f_H/2$.

Another example of the equatorial magnetospheric v.l.f. emissions is shown in Fig. 2, where the emission data, which were observed during the substorm on January 22, 1972, are digitised in 1-min bins and are normalised by the value of f_H computed from onboard magnetometer data. Shown in the upper portion of Fig. 2 is the magnetic latitude of the satellite at the time of the observations. The dark and light shades correspond to the electrostatic mode and the whistler mode emissions, respectively. The frequency of occurrence graph, on the right, shows that the whistler mode has a bimodal frequency distribution with a sharp dip at exactly $f_H/2$.

The variations of the differential electron intensities ($\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{keV}^{-1}$) for the same date are shown in Fig. 3 for electrons of 90° pitch angle and for electrons with the smallest observable pitch angle (close to 25°). The difference between these two plots for each energy is a manifestation of the pitch-angle anisotropy and is indicated by the vertical hatched lines.

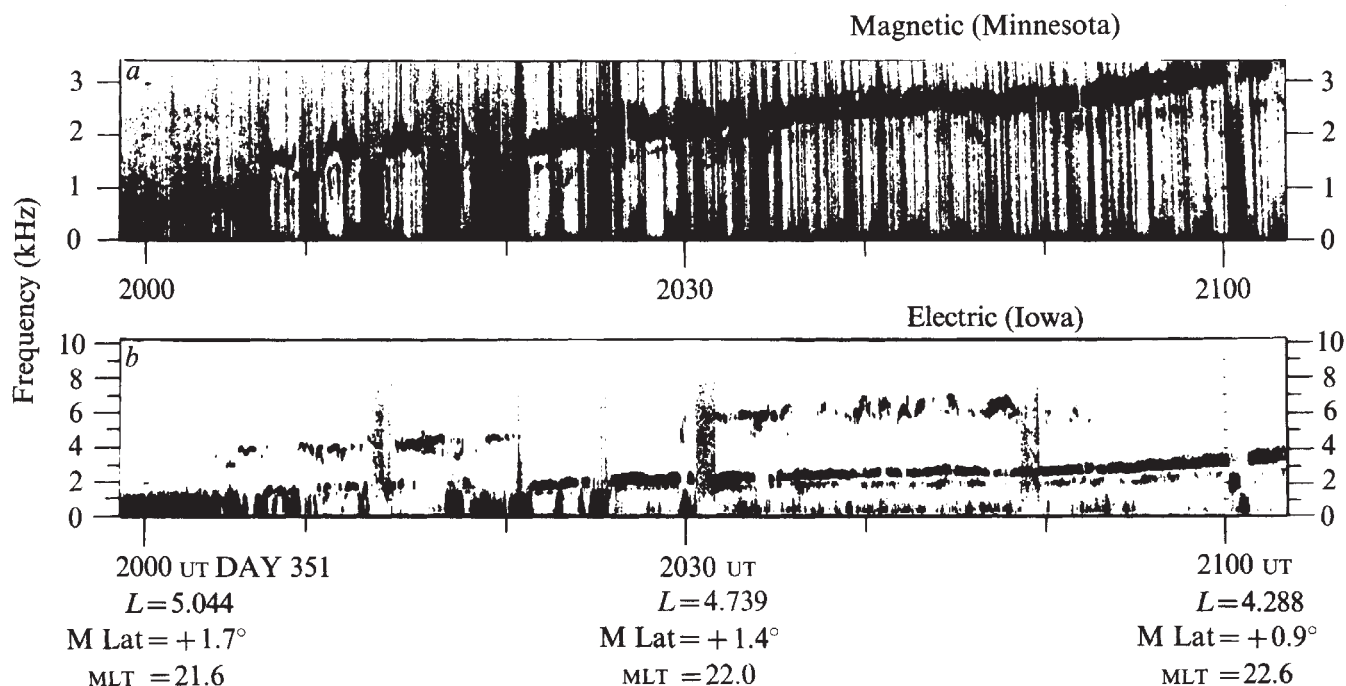


Fig. 1 A part of the v.l.f. emission event during the main phase of the geomagnetic storm on December 17, 1971. The emission of an a.c. magnetic field ($f < 3$ kHz) and an a.c. electric field ($f < 10$ kHz), are indicated in (a) and (b) respectively. The numbers shown at the bottom are: (1) the universal time, UT; (2) a measure of geocentric distance of the satellite in the equatorial plane, L (in R_E units); (3) the magnetic latitude, M Lat; (4) the magnetic local time, MLT. The electron gyrofrequency, f_H , changes from 3,067 kHz at 2000 UT to 3,662 kHz at 2030 UT and to 5,986 at 2100 UT.

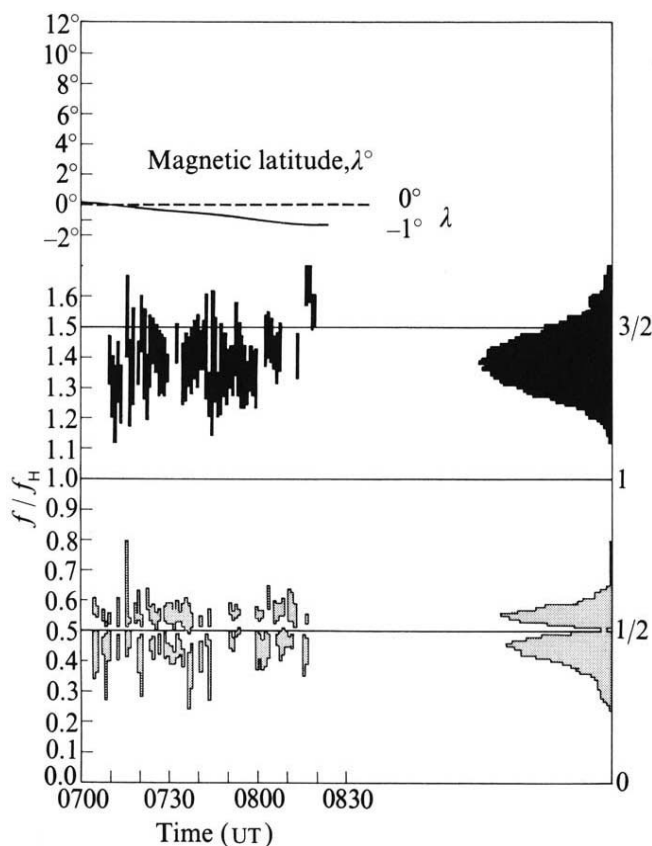


Fig. 2 The frequency distribution of the v.l.f. emissions observed by the S³-A satellite during the substorm on January 22, 1972. The frequency distributions made by accumulating the number of occurrence in each frequency interval ($\Delta f/f_H = 0.01$ is used) are shown on the right side of the figure. There is a conspicuous dip in the bimodal frequency distribution of the whistler-mode emission at exactly $f_H/2$.

The times indicated by horizontal hatched lines and grey shade are the periods when the emissions shown in Fig. 2 were observed and when the satellite is inside the plasmasphere, respectively. A short period around 0700 UT (approximately 2000 MLT—inside the plasmasphere) represents the double structure of the evening side plasmapause².

We can see that the v.l.f. emissions are observed only when the satellite is outside the plasmasphere and that the beginning of the emission coincides with the satellite's encounter with the large electron fluxes there. It should be noticed, however, that the increase in the electron intensities associated with this observed v.l.f. emission is limited to the low energy electrons only (< 6 keV for this event).

To see the energy spectrum of electrons, the flux spectrum at 0730 UT on January 22, 1972 is plotted in Fig. 4. The mean quiet period spectrum observed by this satellite, which has been given by Lyons and Williams¹⁰ is also plotted for comparison. Approximating these energy spectra and the pitch angle distribution of electrons by

$$j(E, \alpha) \sim E^{-n} \sin^m \alpha \quad (1)$$

The parameters n and m for three energy regimes are also shown in Fig. 4. We can see that not only the intensity increase but also the anisotropy is larger in the lower energy electrons.

The growth rate of waves excited by resonant electrons increases with increasing anisotropy of the electrons, and the peak of emission shift to higher frequency as the anisotropy of the resonant electrons increases, particularly for the soft spectrum electrons. For electrons obeying equation (1), the peak of emission is $> f_H/2$ for $m \gtrsim 2$ with $n \gtrsim 1$ (ref. 11). The emission above $f_H/2$ can be also excited by the cyclotron instability of a plasma composed of thermal and quasithermal (20–50 eV) particles if the anisotropy of the quasithermal plasma is suitably large¹². The S³-A satellite did not, however, carry detectors for these low energy plasma particles.

Since the resonance energy, E_R , for electrons encountering the

whistler waves of frequency, f , is given by

$$E_R = E_B \frac{f_H}{f} \left(1 - \frac{f}{f_H}\right)^3 \quad (2)$$

where

$$E_B = \frac{B^2}{8\pi N} \quad \text{or} \quad E_B = 250 \left(\frac{f_H}{f_p}\right)^2 \quad (\text{keV}) \quad (3)$$

B , N and f_p are the magnetic field intensity, plasma density (cm^{-3}) and plasma frequency (kHz), respectively, the energies of electrons resonating with the whistler waves of frequency $f > f_H/2$ are always smaller than those for $f < f_H/2$. In this sense, the large electron intensities shown in Figs 3 and 4 are able to excite only the emissions of higher frequencies, $f \gtrsim f_H/2$.

Fig. 3 Variations of the directional differential electron intensities ($\text{cm}^{-2} \text{s}^{-1} \text{sr}^{-1} \text{keV}^{-1}$) on January 22, 1972. The two lines for each energy correspond to the intensities at pitch angle 90° ($\times$) and of the smallest observed pitch angle (close to 25°) ($\bullet$).

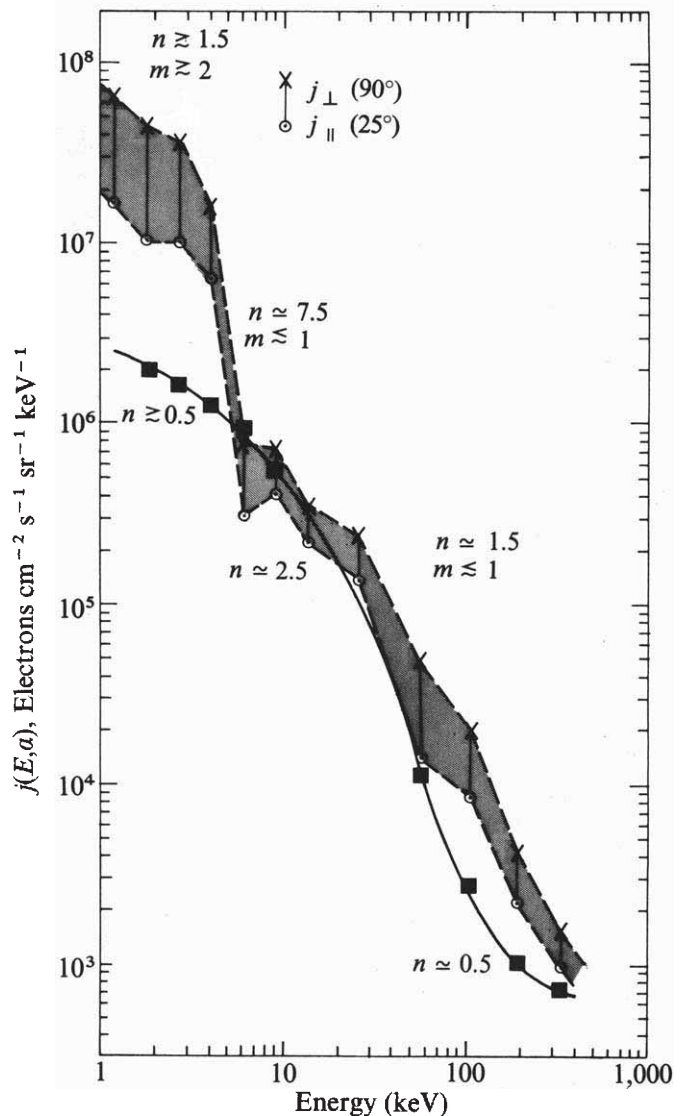
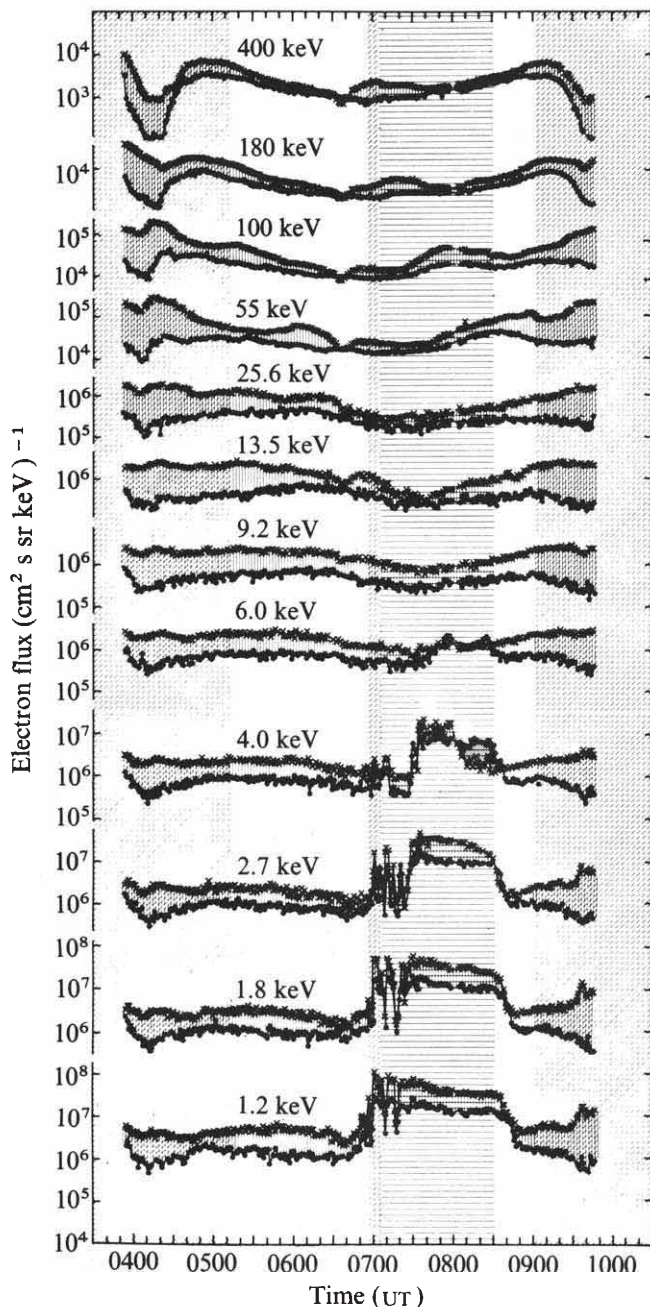


Fig. 4 Differential energy spectra of the enhanced ring current electrons during the v.l.f. emission event compared with the mean quiet time spectrum ($\blacksquare$). The shaded part between two directional intensities ($\times$ for 90° , $\circ$ for 25°) shows the anisotropy of the increased electron intensities at different energies. The parameters n and m for three energy regimes are also indicated in the respective parts of the curves.

On the other hand, because of the $\sim 11^\circ$ tilt of the Earth's magnetic dipole axis from the rotation axis and the small inclination ($\sim 4^\circ$) of the S³-A satellite orbit, emission events are occasionally observed off the equator. Figure 5 shows one example which was observed during the substorm on January 18, 1972. The notation is the same as in Fig. 2, and as indicated by the line in the upper left, the magnetic latitude of the satellite during the event was $> 10^\circ$.

From this figure, we can see that the frequency distribution $> f_H/2$ differs significantly from those of the near equatorial events, indicating that the off-equator propagation of this $> f_H/2$ magnetospheric emission is non-ducted. On the other hand, the emission $< f_H/2$ is similar to that observed near the equator. It seems therefore that these waves are duct-propagated with an upper cutoff of $f_H/2$, which was known from the investigation of whistler propagation in both ground and satellite observations¹³⁻¹⁵.

Based on the information described above, the formation of the bimodal frequency distribution of the equatorial whistler mode emissions with a sharp dip at $f_H/2$ can be explained as follows: (1) The emission $> f_H/2$ is produced near the equator by

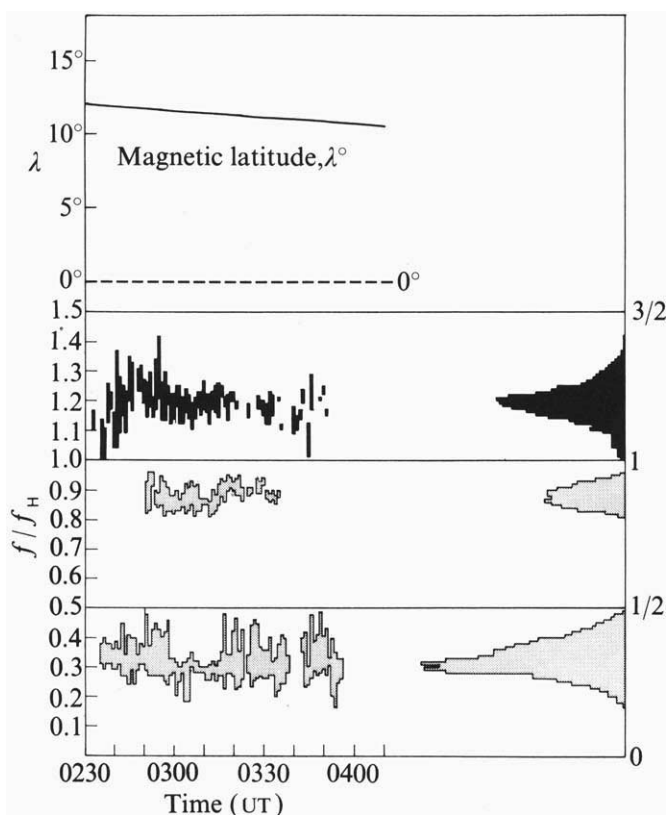


Fig. 5 The normalised frequency distribution. In this off-equatorial emission event, the emissions $> f_H/2$ are weaker than the emissions $< f_H/2$ and the frequencies are quite different from the near equatorial results shown in Figs 1 and 2.

the enhanced ring-current electrons, resonating with the weak broad band whistler-mode noises which are found quite generally in the region of $3 \leq L \leq 10$ of the Earth's magnetosphere; (2) the emissions $< f_H/2$ are produced in a similar manner near the equator but slightly outside the observing location, where the magnetic field is weaker, the corresponding gyrofrequency, f_H' , is significantly smaller than that at the observing location, f_H ; (3) those locally produced emissions with $f > f_H'/2$ propagate unducted off the equator in the magnetosphere, and are reflected back towards the equator from the regions where the lower hybrid resonance frequency, f_{LHR} , exceeds the frequencies of

these waves¹⁶, as depicted schematically in Fig. 6; (4) the reflected waves propagate toward the equator in the narrow density-enhanced ducts whose upper cutoff is $f_H/2$ (refs 13–15).

Since $f_H' < f_H$, we can find, for example, that the emission produced at a frequency $f \approx 0.55 f_H'$ (that is, $f > f_H'/2$) arrives at the observing location with $f = 0.45 f_H$ (that is, $f < f_H/2$), as indicated in Fig. 6.

This idea for the formation of the bimodal frequency distribution with a dip at $f_H/2$ (which is also called "the band of missing emissions" in geomagnetic equatorial whistler emissions, such as chorus^{1,2,6}) has other support. Data gathered by the OGO-3 satellite when crossing the geomagnetic equator on January 21, 1967 (ref. 17), are shown in Fig. 7. The magnetic latitudes corresponding to Fig. 7a, b, c are $\sim 0^\circ$, -2° and -5° , and the arrow on the left side of each indicates the frequency corresponding to $f_H/2$. From these figures, we can see that: (1) emission at $f > f_H/2$ is stronger and more continuous than at $f < f_H/2$, indicating that the emission at $f > f_H/2$ is observed rather close to its source; (2) emission at $f < f_H/2$ is striated, indicating that this emission propagates in narrow ducts of enhanced ionisation; (3) there is no coherence between the upper and lower frequencies, except for the bottom example, (which is rather non-equatorial), indicating that the sources of those two are different, or at least separated.

Further aspects of our theory, including the growth rate of the emissions based on the observed large low energy electron intensity and anisotropy, and the ray tracing of non-ducting emissions produced near the equator with frequencies $f > f_H/2$, are being studied. Finally, there remain effects which are not well understood in the formation of the missing emission band carried by the interference of two modes of propagation and by the Landau damping for a frequency $\sim f \lesssim f_H/2$ (refs 6, 11 and 17). Investigations on the relation between the whistler-mode emission and the electrostatic-mode emission might produce more information about these problems.

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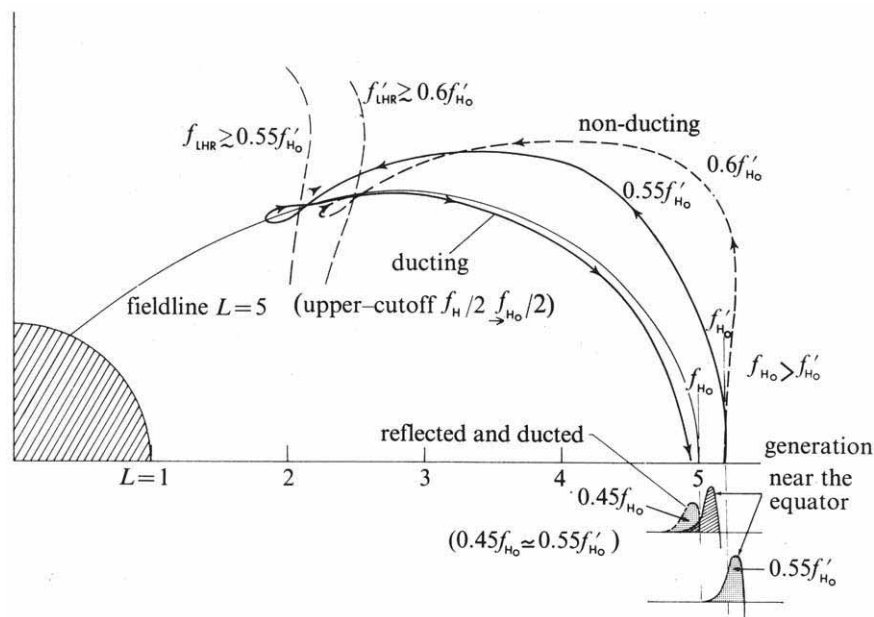


Fig. 6 A schematic depiction of the formation of the bimodal frequency distribution in the magnetospheric v.l.f. emissions observed near the equator. (All ray paths are outside the plasmasphere and the loops of the propagation path at reflection points are exaggerated.)

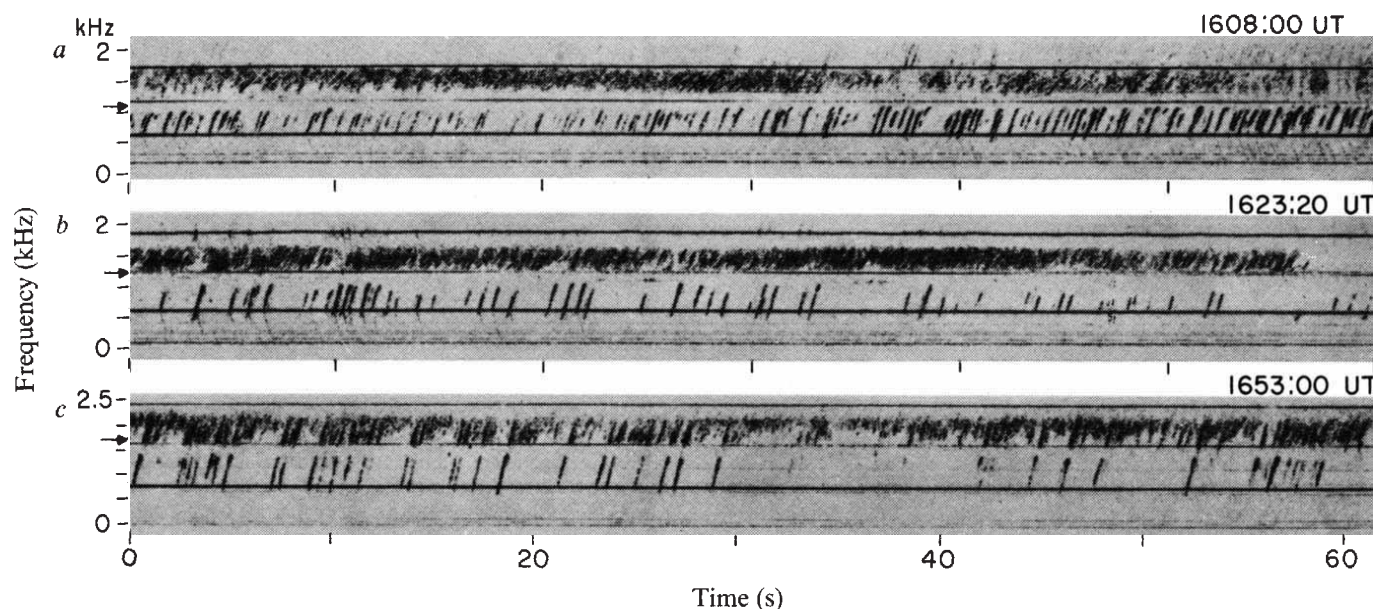


Fig. 7 A portion of the v.l.f. emission data obtained by the OGO-3 satellite along its inbound crossing of the magnetic equator on January 21, 1967. The magnetic latitude of the three records is (a) 0° , (b) -2° and (c) -5° , respectively. The arrows on the left side of the figure indicate the frequency of $f_H/2$. (Reproduced from ref. 17 by the courtesy of Professor R. A. Helliwell.)

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Gas movement through sea ice

UNLIKE ices from pure or freshwater, sea ice is a highly permeable medium for gases. We have found that: (1) the migration of these gases along the grain boundaries (probably in brine channels) was 2 to 6 times as great as that at right angles to the principal axis of the grain boundaries; (2) the rate of penetration is $\sim 30 \text{ cm h}^{-1}$ at -15°C for halogenated gases (in nl ml^{-1} quantities) and 60 cm h^{-1} at -7°C for carbon dioxide (in $\mu\text{l ml}^{-1}$ quantities); (3) the vertical migration is about twice as fast as horizontal migration at -15°C , and (4) for one experiment with a block of semi-fresh pressure ridge ice, the migration rate was $\sim 60 \text{ cm h}^{-1}$ at -15°C . The permeation constants are estimated to be $10^{-7} \text{ cm}^2 \text{ s}^{-1} \text{ atm}^{-1}$ at -15°C for SF_6 , and $2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \text{ atm}^{-1}$ for CO_2 at -7°C . These data strongly indicate that gas migration through sea ice is an important factor in ocean-atmosphere winter communication particularly when the surface temperature is $> -10^\circ \text{C}$.

It has been correctly reported¹ that pure water and lake water ice are effective barriers against any significant migration of gases at temperatures $< -0.5^\circ \text{C}$. Their permeabilities are 10^6 less than that of carbon dioxide through water, with lake water ice an order of magnitude more permeable because of traces of impurities in the ice. This is understandable when one considers results^{2,3}, which show that the size of veins at three grain intersections open or close drastically within a few hundredths to a few tenths of a degree below the freezing point for freshwater ices.

On the other hand, large brine loads would extend the temperature range over which these vein and brine channels would remain open to several degrees below the freezing point. The sea-ice temperature, of course, will be a gradient from -2°C at the water-ice interface to that of the prevailing atmosphere at the air-ice interface.

We also observed brine to enter bore holes in noticeable quantities after 12 h at -15°C : at -10°C it was only slightly faster. At -6.5°C brine temperatures, however, bore holes filled to the hydrostatic level within an hour. The bore holes were 50–75 cm deep in 2-m thick ice. Ink in brine was observed to travel 30 cm vertically within 30 min through a freshly quarried block of sea ice at -7°C . There are also results^{4,5} on the flow of water through ice at temperatures close to 0°C .

Tracer gases (sulphur hexafluoride, perfluoromethyl bromide, and perfluoroethyl bromide) were resolved and detected in an electron capture gas chromatograph with a short Porapak N column. Carbon dioxide was analysed with a thermal conductivity device. Experiments with shorefast annual ice using halogenated tracer gases were carried out in April at the Naval Arctic Research Laboratory (NARL) at Barrow, Alaska, and other *in situ* experiments in pack ice were performed at the Arctic Ice Dynamic Joint Experiment (AIDJEX) Camp Caribou located ~ 240 miles NE of Barrow. Both sets of experiments provided the same rate values at -15°C . Another set of *in situ* experiments at NARL in May, when the temperature was -7°C , provided the data for CO_2 . In early experiments, bore holes were charged with one or more of the halogenated tracer gases, and then the top of the hole was covered with a metal plate which was sealed to the surface with fresh water and snow. At intervals, cores were taken beside the sealed holes, leaving 25–30-cm ice walls. The cores were taken in the plane of the predominant ice grain boundaries from the gas-spiked holes, and perpendicular to it. These cores were immediately placed in gas-tight containers made from glass sewer pipe, and then flushed with He by means of stopcock fittings on the ends of the containers. In the laboratory, large loads of tracer gases were found in the head space gas from cores taken ~ 1.5 h after the start of the experiments. For all other experiments, 5-cm diameter bore holes were drilled to a depth of up to 1 m in the ice outside the work hut. Ice walls of 30–90 cm were left between three vertical holes. Metal plates with swagelock fittings were sealed to the ice over these holes with snow and freshwater. The two outside holes were manifolded through a closed

loop pump and switching system so that samples could be valved directly into the gas chromatograph. In other experiments, diagonal holes were bored in the ice so that 10- and 20-cm ice walls had to be penetrated vertically by the gases.

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On the marine geochemistry of cadmium

STUDIES of the geochemistry of trace metals in sea water are of great potential importance, given the wide diversity in their properties. Knowledge of their distribution provides information not only about the specific chemical processes in which they are involved but also about the formation, destruction and diagenesis of the major solid phases in which they become incorporated by biological and authigenic processes. Previous efforts to elucidate the behaviour of cadmium in the ocean have produced no systematic correlations with other oceanographic properties¹. At the low levels encountered (< 0.1 parts per 10^9), problems with analysis and with contamination during sampling can be severe. Recent advances in analytical and handling techniques have led to progress in our understanding of other trace metals^{2–4}, hence a re-examination of the marine geochemistry of cadmium is now appropriate. This note describes the general distribution of cadmium in the ocean, based on three detailed vertical profiles from the Pacific.

Fig. 1 Cadmium, phosphate, and silicate profiles for three stations in the Pacific Ocean. ○, Silicate; ●, phosphate for (a) Stn 219 (b) Stn 226 and (c) Stn 293.

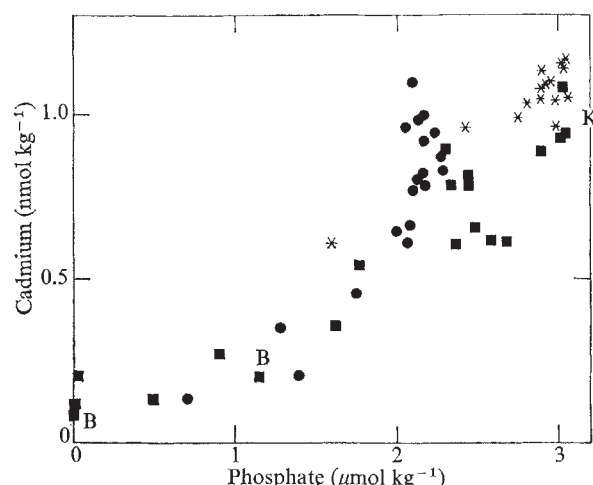
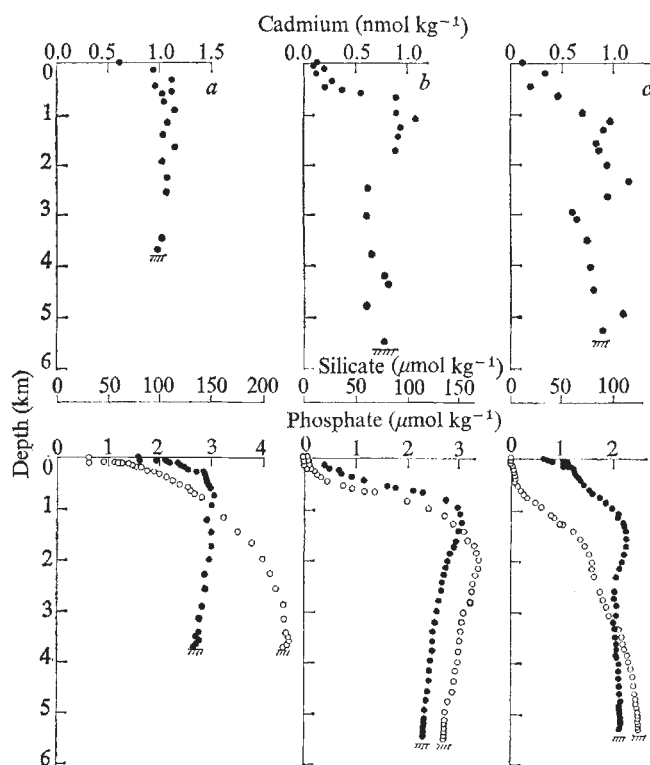


Fig. 2 Cadmium concentration against phosphate for three Pacific profiles (●, Stn 293; *, Stn 219; ■, Stn 226) and three data points from the literature, B (ref. 4) and K (ref. 6).

Station locations were chosen to cover a broad range of oceanographic conditions, from the south-western Pacific, S of New Zealand (Stn 293) to the temperate mid-Pacific, E of Japan (Stn 226) and the North Pacific upwelling region in the Bering Sea (Stn 219) (Table 1). The samples were taken using rosette-mounted 30-l PVC Niskin bottles with epoxy-coated internal springs and stored in acid-leached polyethylene containers, after acidification to $pH \approx 2$. The handling of containers and samples is described in detail elsewhere³. Cadmium was preconcentrated from 100-g samples by cobalt pyrrolidine dithiocarbamate coprecipitation⁵ and analysed by atomic absorption spectrometry using a heated graphite atomiser (E. A. Boyle and J. M. Edmond, unpublished). The estimated precision and accuracy of the analysis is ± 0.1 nmol kg^{-1} . The cadmium data and relevant hydrographic information are listed in Table 1.

Depth profiles of cadmium are shown in Fig. 1 along with profiles of the nutrients phosphate and silicate. It is readily apparent that cadmium is depleted in the surface ocean relative to the deeper waters. Such a distribution indicates uptake by organisms at the surface and regeneration from sinking biological debris deeper in the water column. The range in concentration is over a factor of at least ten, from 0.1 ± 0.1 to 1.1 ± 0.1 nmol kg^{-1} .

The cadmium profiles resemble those of phosphate more than silicate: the concentrations increase rapidly with depth to a maximum at ~ 1 km and then decrease slightly below this. The covariance with phosphate is consistent for all stations (Fig. 2) and suggests that cadmium is regenerated in a shallow cycle, like the labile nutrients, rather than deeper in the ocean, as is silicate.

The results are compatible with some recent cadmium data. Bender and Gagner⁴ report values for the Sargasso Sea; surface (0.05 nmol kg^{-1}) and deep (0.30 nmol kg^{-1}): Knauer and Martin⁶ give a value for the 1,000-m depth near Hawaii (1 nmol kg^{-1}) and report surface concentrations below their detection limit (0.2 nmol kg^{-1}). Estimates of the phosphate concentrations at these locations were made using data from nearby GEOSECS stations, and are plotted in Fig. 2. The agreement is excellent. The cadmium-phosphate correlation observed in these stations probably holds throughout the world ocean.

A detailed comparison of the chemical composition of plankton with the hydrographic correlation of cadmium and phosphate is not possible because few analyses include both elements. Goldberg *et al.*⁷ report analyses of *Sargassum* and planktonic animals with Cd/P molar ratios of 4×10^{-4} and 6.5×10^{-4} respectively. These values are close to the water column ratio 3.5×10^{-4} . Martin and Broenkow⁸ observed

Table 1 Cadmium and associated hydrographic data from three Pacific GEOSECS Stations

Depth (m)	Potential temperature (°C)	Salinity (‰)	Silicate (nmol kg ⁻¹)	Phosphate (nmol kg ⁻¹)	Cadmium (nmol kg ⁻¹)
Station 219 10/8/73 177°17.5'W, 53°6.6'N					
5	7.107	33.069	32.2	1.60	0.61
160	3.533	33.524	74.2	2.42	0.96
349	3.548	33.886	100.8	2.89	1.13
460	3.417	34.027	113.7	2.97	0.96
599	3.294	34.159	128.0	3.03	1.14
642	3.226	34.177	131.0	2.89	1.04
792	3.047	34.266	141.9	2.89	1.05
943	2.834	34.337	150.9	3.04	1.16
1,189	2.531	34.413	163.3	2.95	1.09
1,440	2.249	34.480	176.5	3.05	1.05
1,677	1.983	34.536	190.1	3.01	1.15
1,988	1.741	34.585	202.7	2.98	1.04
2,287	1.594	34.613	209.5	2.91	1.08
2,583	1.482	34.635	214.6	2.89	1.08
3,480	1.310	34.665	226.9	2.80	1.03
3,711	1.281	34.669	221.7	2.74	0.99
Station 226 11/9/73 170°36.5'E, 30°34.0'N					
8	24.73	35.055	3.3	0.02	0.12
36	24.75	35.053	2.7	0.01	0.09
82	23.58	35.005	3.2	0.02	0.20
207	15.19	34.627	7.2	0.49	0.13
382	12.01	34.396	16.0	0.89	0.27
456	10.33	34.277	23.8	1.15	0.20
555	7.92	34.103	38.5	1.61	0.36
591	7.28	34.066	45.6	1.76	0.54
687	5.62	34.031	68.5	2.29	0.89
982	3.62	34.265	121.6	2.97	0.89
1,126	3.20	34.371	135.3	3.02	1.08, 1.06
1,274	2.85	34.440	145.0	3.03	0.96, 0.89
1,421	2.53	34.488	153.7	3.00	0.92
1,712	2.06	34.560	165.1	2.89	0.88
2,442	1.48	34.639	163.5	2.67	0.61
3,005	1.28	34.663	154.7	2.58	0.61
3,785	1.141	34.679	149.7	2.47	0.65
4,175	1.095	34.685	147.9	2.43	0.78
4,361	1.075	34.684	145.7	2.43	0.81
4,779	1.03	34.689	139.9	2.36	0.60
5,446	0.971	34.695	136.2	2.33	0.78
Station 293 3/1/74 178°5.0'W, 52°40.0'S					
3	11.328	34.419	2.7	0.72	0.13
216	7.65	34.419	5.2	1.28	0.31, 0.38
458	6.46	34.342	6.6	1.40	0.20
685	5.27	34.268	17.6	1.76	0.46
961	4.07	34.312	30.8	2.00	0.71, 0.73
1,143	3.30	34.347	44.8	2.13	0.98
1,290	2.83	34.395	53.8	2.24	0.91
1,587	2.45	34.525	68.9	2.28	0.83
1,733	2.37	34.577	73.4	2.27	0.87
2,029	2.19	34.659	80.3	2.19	0.78
2,326	2.018	34.708	83.7	2.10	1.07, 1.12, 1.29
2,621	1.78	34.732	88.5	2.06	0.96
2,771	1.65	34.763	91.9	2.06	0.61
3,065	1.422	34.735	98.3	2.08	0.66
3,509	1.05	34.723	108.7	2.10	0.77
4,019	0.77	34.711	116.6	2.13	0.80
4,474	0.586	34.705	120.5	2.15	0.82
4,926	0.48	34.700	124.5	2.16	0.98, 1.26
5,271	0.446	34.699	125.3	2.17	0.92

cadmium enrichments by a factor of 3 in plankton from the Baja California upwelling region relative to organisms in the open Pacific. Cadmium, like phosphate, is enriched in the surface waters of upwelling regions (for example, Stn 219) relative to areas of lower productivity. The phosphate content of the Baja California waters is about a factor of 3 higher than further away from the coast (R. C. Dugdale and M. L. Healy, unpublished; F. R. Richards, unpublished), and a similar enrichment factor should apply to the cadmium concentration in these waters. It is evident that the cadmium content of plankton depends on the concentration in the water in which the organisms grow. A mechanistic interpretation of the cadmium-phosphate correlation requires information on the site of incorporation of cadmium in organisms and sinking biogenic debris. Similar depth profiles would result if cadmium were carried in either the soft tissues of organisms or in the more

rapidly dissolving hard parts, such as celestite (SrSO₄) (ref. 9). Future work on the chemical composition of marine plankton should attempt to establish the location of the various elements in the organisms.

There is little information on the cadmium content of river waters. Where measurements have been attempted, concentrations were often near or below the detection limits of the instrumentation (ref. 10 and refs therein). To derive an estimate for the continental supply rate of this element, a glass fibre-filtered sample from the mouth of the Amazon River was obtained at Macapa, Brazil in May 1974. The cadmium concentration of this sample (0.6 nmol kg⁻¹) may be tentatively taken as representative of world rivers. The resulting estimate of the oceanic 'residence time' of cadmium is ~ 50,000 yr.

Last, it should be noted that there is no evidence in these data for cadmium pollution in the open ocean. Indeed, the

efficient scavenging of this metal by organisms will rapidly remove pollutant cadmium from the mixed layer so that it should not be expected to accumulate in the surface ocean.

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Combustion sources of atmospheric chlorine

ESTIMATES of the primary sources of tropospheric gaseous chlorine compounds (ClX) which have the potential for reaching the stratosphere, list man-made halogenated hydrocarbons (such as refrigerants and aerosol propellants), man-made and natural hydrogen chloride sources (such as burning of waste materials and volcanic activity), sea spray and naturally occurring chlorinated hydrocarbons. The existence of an unidentified source of tropospheric ClX has been proposed¹⁻⁴ which has since been postulated to be combustion. The purpose of this paper is to examine and estimate the strength of fires as ClX sources.

Chlorine, as well as the other halogens, serve as free-radical traps in flames and are effective in suppressing flaming combustion and promoting glowing combustion⁵. When chlorine compounds are heated in contact with cellulose materials, ClX is produced either by reactions involving the hydroxyl groups of the cellulose, or by reactions involving 'active' water formed *in situ* by dehydration⁶. About 1% of the combustion products evolved from wood is methane⁷, which can be converted to methyl chloride by the chlorine available in the wood. Bethge and Troeng⁸ found that the natural chlorine content of wood pulp and other cellulosic materials ranged from 0.07 to 2.68 mg g⁻¹, that the chlorine content of wood was greater by an unspecified amount than that of unbleached wood pulp, and that 86% of the chlorine content of the sample was lost during combustion.

Lovelock (personal communication) refined his earlier estimate¹ to 1 cm³ of methyl chloride gas produced for each gram of cellulose burned in glowing combustion. This is approximately equivalent to 2.2 mg of methyl chloride or 1.6 mg Cl produced per gram of cellulose burned. If the 86% loss of Cl during burning is representative, the average initial chlorine level in cellulose is 1.8 mg g⁻¹. There is good agreement between experiments, excepting those which involved flaming combustion.

The common combustion products of most burning plastics are smoke, CO, CO₂ and H₂O vapour. Trace amounts of extremely complex liquid, solid and gaseous combustion

products also occur⁹. PVC is different from other plastics because its principal combustion product is HCl (refs 10, 11). The overall chlorine content of PVC is ~ 49%, varying with the amount of various pigments and lubricating organic additives. Yields of HCl during thermal decomposition vary from almost complete (583 of a possible 584 mg (ref. 12)) to ~ 95% (ref. 10).

PVC is widely used for many purposes and consequently is usually involved to some extent in any man-made fire. The most reliable estimates of PVC production in the USA are given in Table 1. In 1974, 6.5% of this production went into various forms of packaging materials¹³, which are ultimately disposed of as waste. As a result of Executive Order 507, much of the municipal waste and waste from suburban and rural areas in the USA is now buried. Some of the PVC is burnt in free-burning fires such as those in fireplaces and home incinerators. Much of the HCl produced in these fires does not reach the atmosphere because it is quickly deposited on adjacent surfaces, is mixed with smoke particles (both solid and liquid) and is absorbed by the water produced during combustion. An indication of the reactivity of HCl and its presence in smoke from the burning of waste is the heavy corrosion of the metal parts of municipal incinerators and the observed inhibition of combustion of municipal wastes¹⁴. I estimate that ~ 50% of the packaging material was burnt in 1965, decreasing linearly to 10% in 1974, and that 10% of the evolved Cl is converted to methyl chloride and other chlorinated hydrocarbons. Table 1 shows that the production of methyl chloride from waste has only recently declined.

Disposition of the rest of the PVC in the USA apparently is not available. English estimates¹⁵ are that 50% of the PVC produced goes into construction. The National Fire Protection Association estimate of the loss rate of new construction because of fire is \$0.59 per \$100 or ~ 0.6% yr⁻¹ (ref. 16). Thus, ~ 0.3% of the PVC produced yr⁻¹ is burnt in urban and industrial fires and ~ 10% of this amount is converted to chlorinated hydrocarbons. As shown in Table 1, the total man-made production of methyl chloride is continuing to rise, in spite of the reduction in the burning of waste.

The area burnt in wildfires is well documented¹⁷. In 1974, 537,020 ha were burnt in the small wildfires. The amount of fuel per unit area (fuel loadings) was quite variable ranging from 1 to 2,500 t ha⁻¹. Estimates derived from wildfire data (O. L. Holmes, US Forest Service, R-5, personal communication) and other sources¹⁸⁻²¹ indicate that the average fuel loading is ~ 30 t ha⁻¹. Of this, ~ 40% is fast burning fine material; the residual is coarse material which tends to burn by glowing combustion.

Controlled burning encompasses a large range of activities including forest residue reduction, agricultural waste burning and other mild fires. The weather and fuel conditions for these fires are deliberately chosen to promote low flames and glowing combustion to minimise fire intensity, and to reduce the probability of fire escape. Much of the agricultural residual materials have a high mineral content (for example, rice straw 15%, sugar cane 30%) which also promotes glowing combustion and increases the production of methyl chloride.

Yamate's²² survey of the burning of forest and agricultural residues in the United States is in agreement with other sources¹⁹⁻²¹ except for forest residue reduction in the Pacific North-western States which is 424 times greater. The Forest Service was taken as the authoritative source. All values in Table 2 of the amount of fuel burnt in forests have been decreased by 40% to account for the rapid burning of fine fuels.

It is generally accepted by observers of large fires that the convection columns reach altitudes of 10 km or more²³. Cramer of the US Forest Service (personal communication) measured the tops of the 1933 and 1939 Tillamook, Oregon, fires at 12 and 13 km. The convection column above the 1967 Sundance Fire in Montana reached 10.5 km and was carried to 13 and 14 km by the isentropic flow field downwind²¹.

Table 2 Methyl chloride from combustion sources in the USA, 1972-74

Source	Area burnt (ha)	Fuel loading (t ha ⁻¹)	Fuel burnt (t yr ⁻¹)	Methyl chloride (t yr ⁻¹)	Totals (t yr ⁻¹)
Agricultural					
Field residues	1,767,771	1.13	2.00 × 10 ⁶	2,700	2,700
Forests					
Forest residues					
Pacific North-west	Not currently available		39.4 × 10 ⁶	52,000	
California	Not currently available		0.9 × 10 ⁶	1,200	
Southern States	8.10 × 10 ⁵	25	20.0 × 10 ⁶	26,700	
Rest of USA	8.50 × 10 ⁵	3	2.5 × 10 ⁶	3,300	83,200
Wildfires (A, B, C, D, E)	4.69 × 10 ⁵	30	14.1 × 10 ⁶	18,600	
Wildfires (F, G)	2.29 × 10 ⁵	30	18.9 × 10 ⁶	24,900	43,500
Man-made (Table 1)					80,600
Totals					210,000

These large fires may spread at rates up to several thousand ha h⁻¹. Fuel loadings vary from one to as much as 1,250 t ha⁻¹. Combining the fire spread rates with the heat of combustion of wood (~ 5,000 calorie g⁻¹) gives an energy release rate of 10⁹-10¹² calorie s⁻¹. Such energy release rates are as great as the largest natural thunderstorms²⁴. The primary energy source is locally intensive at the ground rather than in the extensive of the cloud as in a thunderstorm. Studies of convection columns²⁵ show that most of the air passes through or near the bottom of the column in or near the combustion zone. There is a little evidence of lateral entrainment into these convection columns at heights > 100 m above the fire. Condensation within the convection column is apparently determined by the lifting condensation level of the ambient air at the ground level. This type of fire usually occurs in extremely dry conditions and, if condensation occurs at all, the level is > 6,000 m.

Analysis of the Project Flambeau convection column²⁵ showed that these large fires followed a consistent pattern, and that a fire had to have at least 27 ± 2 hectares burning (not burnt) to be classified as a mass fire. The application of the Project Flambeau results to large wildfires requires an analysis of the geometry of these fires. In large, vigorously spreading wildfires the flame front at the fire's head is ~ 150 m thick in timber and 50 m thick in brush. The shape of the burnt area is a complex function of terrain, wind, humidity, fuel moisture, fuel distribution, percentages of live and dead materials and other factors. A variety of simple models can be assumed, but the simplest is a square, of side 2r, with two semicircles of radius r at each end. The active fire front is of radius r and of thickness d which approximates the final form of the Sundance fire¹⁷.

The total area burnt A_B is the sum of the end semicircles and the centre square

$$A_B = \pi r^2 + 4r^2 \quad (1)$$

The significant burning area, A_s , is

$$A_s = \pi (2rd + d^2)/2 \quad (2)$$

Solving equation (2) for r and substituting into equation (1) gives the area burnt during a mass fire as

$$A_B = (\pi + 4)[(A_s/d) + (d/2)]^2 \quad (3)$$

Substituting the characteristic values for d gives minimum sizes of $A_B = 4.09 \times 10^6$ m² for timber and $A_B = 4.16 \times 10^7$ m² for brush.

Fires this size are categorised as class F (1,000 to 4,999 acres) and class G (> 5,000 acres) in the USA where there were 227 class F and 53 class G wildfires in 1974 (ref. 17). These fires burnt a total of 628,605 ha (1,552,654 acres), which is a normal yearly area. The flame front primarily burns the fine fuels which average 12 t ha⁻¹. About 50% of the remaining fuel, or 8 t ha⁻¹, is later burnt in glowing combustion and produces methyl chloride. This air is entrained into the convection column and transported to at least tropopause levels. The results of the various production processes are shown in Table 2.

Taking account only of documented fires, I estimate the production of methyl chloride in the USA by burning cellulose and PVC averages 210,000 t yr⁻¹. The requirement that CIX be produced during glowing combustion may be overly stringent and the estimate may be 40% too low. The rate of production by this source has been estimated to be 5.2×10^6 t yr⁻¹ (ref. 26). For instance, current estimates are that half of the timber cut in the world is used for firewood²⁷. Forest fire wood and agricultural burning has been a common practice throughout the world for many thousands of years, swamping the US CIX production. If Eckholm's estimates²⁷ of forest depletion are correct, by the year 2000 there will be a significant decrease

Table 1 Recent US yearly production of polyvinyl chloride and estimated release of methyl chloride

Year	PVC production (Mt yr ⁻¹)*	Methyl chloride produced from PVC burnt in waste (t yr ⁻¹)	Methyl chloride from building fires (t yr ⁻¹)	Total methyl chloride (t yr ⁻¹)
1965	8.006	26,020	24,018	50,038
1966	9.462	28,018	28,386	56,404
1967	9.435	25,212	28,305	53,517
1968	10.04	23,929	30,120	54,049
1969	13.03	27,291	39,090	66,381
1970	13.29	23,996	39,870	63,866
1971	15.58†	23,630	46,740	70,370
1972	19.60‡	24,064	58,800	82,864
1973	20.08§	18,853	60,240	79,093
1974	21.87	14,216	65,610	79,826

* US Tariff Commission Summaries of Trade and Tariff Information Schedule 4, Vol. 7 TC 408, Washington, DC.

† US Tariff Commission Synthetic Organic Chemicals US Production & Sales (1971) TC 614.

‡ Ibid. 1972, TC 681.

§ Ibid. (1973) TC 728.

|| US International Trade Commission, Preliminary Report on US Production of Selected Organic Chemicals (1974).

in CIX production because of lack of wood and cellulose for burning.

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Modelling climatic response to orbital parameter variations

ACCORDING to the astronomical or Milankovitch theory, the glacial fluctuations of the Pleistocene are the climatic response to secular variations in the obliquity, eccentricity, and longitude of perihelion of the Earth's orbit. Variations in these orbital parameters during the past several hundred thousand years can be computed with great precision¹, and the resulting changes in incident radiation at the top of the atmosphere are easily obtained. If one can predict the climatic response to perturbations in incident radiation, one can, therefore, test the Milankovitch theory by comparing the predicted climatic changes with those inferred from the geological record. No other theory of the ice ages admits such a straightforward check on its validity. We have made a preliminary attempt at verifying the Milankovitch theory using a zonally symmetric energy-balance climate model forced with seasonally varying insolation and obtain generally favourable results.

The model predicts, as functions of latitude and time of year, the temperatures of two atmospheric layers (representing the upper and lower halves of the troposphere), surface temperatures over land and ocean, the depth of snow over land, and the sea ice thickness. A radiative transfer model similar to that of Manabe and Wetherald² is used to compute radiative heating rates in the two atmospheric layers and the net flux at the surface. Cloudiness and relative humidity are assumed to be fixed and independent of latitude in the radiative calculation. Heat is transferred meridionally by linear diffusion in the atmosphere.

This crude atmospheric model is coupled to the land and ocean surfaces through simple boundary layers. The ocean temperature is assumed to be that of a 40-m deep isothermal layer of water, and the land temperature that of a water-saturated surface with no heat capacity. No heat is transported by the model's 'ocean'. Ocean temperatures are not allowed to fall below 273 K, at which temperature sea ice is assumed to form. Sea ice thickness and temperature are predicted with a

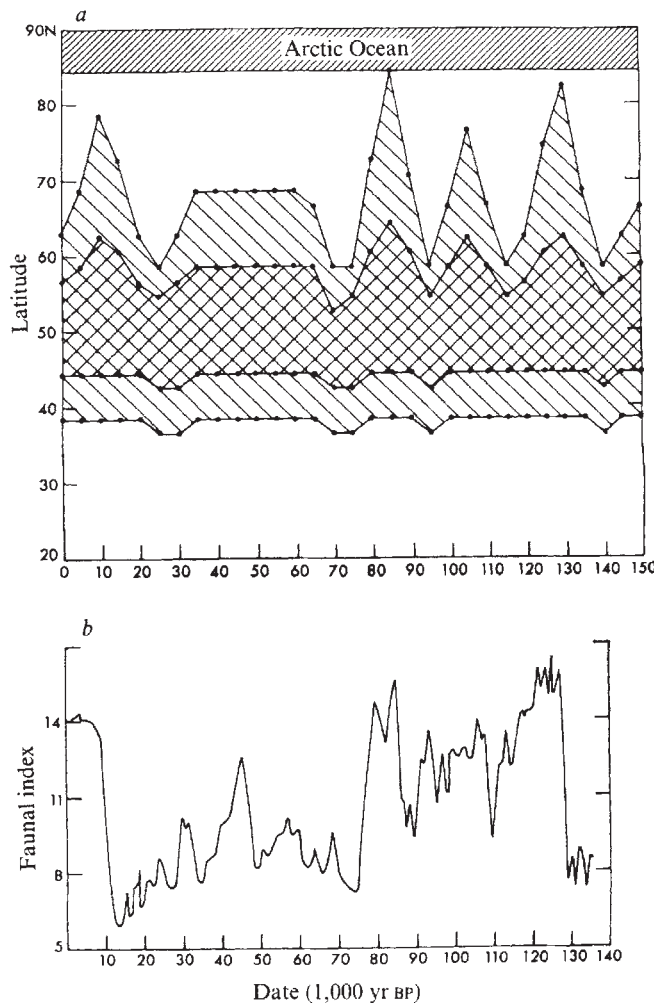


Fig. 1 The model's 'palaeoclimatic record' for the past 150,000 yr is shown in the upper figure. *a*, Four points are plotted for each experiment, corresponding to the seasonal limits of land snowcover (hatching) and sea ice (cross hatching) in the Northern Hemisphere. The northernmost point represents the minimum (summer) extent of snowcover on land. North of this latitude, snow is free to accumulate from year to year; we therefore identify it with the extent of continental glaciers. The other three points (from north to south) represent the minimum extent of sea ice, the maximum extent of sea ice, and the maximum extent of snowcover over land. Changes in snowcover and sea ice extent predicted by the model in the Southern Hemisphere are much smaller than these changes in the north. ('Arctic Ocean' refers to latitudes at which there is no land.) *b*, A plot of palaeotemperatures inferred from faunal abundances (of North Atlantic plankton) in a deep-sea core in the North Atlantic³. Its gross features are consistent with other palaeoclimatic reconstructions of this period. Three warm peaks separated by ~ 20,000 yr mark the last interglacial. The rapid descent into the Wisconsin ice age occurs at ~ 70,000 yr BP, followed by a period of relatively warm climate (middle Wisconsin), then by the late Wisconsin glacial maximum, and finally by the warmth of the Holocene.

scheme very similar to that used by Bryan³. It is also assumed that snow falls at a fixed rate, dependent only on latitude, whenever the surface temperature falls below 273 K. Snowmelt and snowdepth are then computed in a straightforward manner. Surface albedos are chosen to be 0.1 over bare land and open water, and 0.7 over snow-covered land and sea ice. Zonally symmetric atmospheric heating rates are obtained by combining the predicted rates over land and ocean in proportions determined by the fraction of land around a latitude circle.

The model is similar to diffusive energy-balance models used by Sellers⁴ and North⁵, with changes in surface albedos having a dominant role in determining the sensitivity to changes in external forcing.

For a given set of orbital parameters, we compute the flux of solar radiation incident at the top of the atmosphere as a function of latitude and time of year. The model's equilibrium response to this pattern of insolation is then obtained by integrating its equations of motion until a steady state is achieved. The results of such computations for the present orbital parameters and for those at 5,000-yr intervals into the past (for the past 150,000 yr) are summarised in Fig. 1a. Figure 1b (reproduced from Sancetta, Imbrie, and Kipp⁶) is a plot of a faunal index obtained from counts of planktonic foraminifera in a single deep-sea core in the North Atlantic. The index is calibrated to be an estimate of summer sea-surface temperatures in °C.

The largest difference in mean Northern Hemisphere surface temperature between any two of the experiments described in Fig. 1a is 2.4 K. Changes in mean Southern Hemisphere surface temperatures are smaller—at most 0.4 K.

The model is particularly sensitive to insolation anomalies during the Northern Hemisphere summer, with high obliquity (large seasonal variation) and perihelion near Northern Hemisphere summer solstice producing the warmest climates. During the summer, when land surface temperature gradients are small and incident solar radiation is large, changes in temperature produce the largest displacements of the snow margin, and the resulting changes in albedo produce the largest changes in absorption of incident radiation. The Southern Hemisphere, with less land and a smaller seasonal variation, is not as sensitive to the seasonal redistribution of insolation. This sensitivity to summer insolation in the Northern Hemisphere is consistent with Milankovitch's assumptions, and the time evolution predicted by the model is similar to his summer insolation curves for high latitudes in the Northern Hemisphere⁷. The reader is referred to the literature^{8,9} for a detailed comparison of the Milankovitch curves with the geological record.

Large discrepancies between the model and the record of the recent past are evident from Fig. 1. For the present values of the orbital parameters, the model gives a climate intermediate between a predicted glacial minimum at 10,000 yr BP and a glacial maximum at 25,000 yr BP, while observations place the present climate much closer to the most recent climatic optimum at ~ 5,000 yr BP. This discrepancy may arise from other sources of variability, or from a lag resulting from the time required to change the temperatures of abyssal waters or to build and destroy continental glaciers, or to other model deficiencies which may tend to overemphasise the importance of summer insolation anomalies in the Northern Hemisphere.

In spite of such discrepancies, Fig. 1 suggests that a substantial portion of climatic variability on these time scales can be understood as the equilibrium response to perturbations in the orbital parameters. Quantitative tests with more convincing climate models are needed to confirm these very preliminary results. If this theory is correct, the palaeoclimatic record becomes an invaluable source of information on climatic sensitivity.

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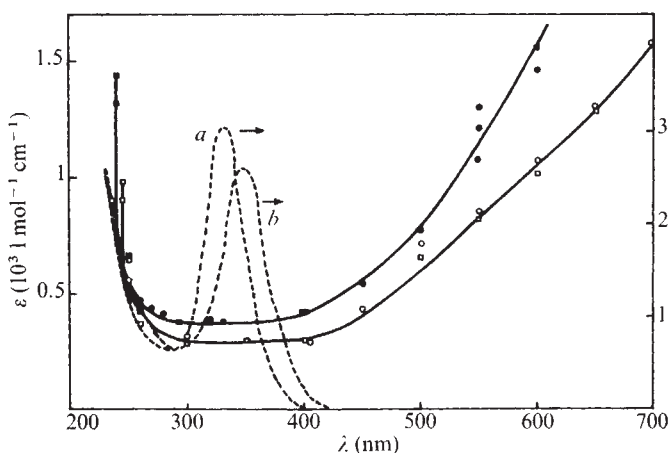
Ultraviolet absorption by metal-ammonia solutions

By pulse radiolysis of aqueous solutions at room temperature, new absorption bands for H, OH, D, OD and e_{aq}^- in the ultraviolet region down to 188 nm have been observed^{1,2} and recently confirmed³. The favoured interpretation was a redshift of the absorption band of water caused by perturbation by the solutes (H, D, e_{aq}^-) similar to the β -band displacement in alkali halide crystals¹. It was also suggested that such a band may exist in liquid ammonia, implying that it is a general phenomenon associated with the solvated electron. Furthermore, the transient spectrum obtained by pulse radiolysis of pure liquid ammonia (−45 °C) shows, in addition to the broad infrared absorption of the solvated electron, two unidentified bands in the ultraviolet region⁴. To study optical properties in this range, we chose metal-ammonia solutions which are relatively stable although their decomposition leads to the amide ion which itself absorbs strongly in the same region. Our findings strongly support the hypothesis, though amide and hydrogen are the only products and have been thoroughly studied.

The solutions were prepared taking care to ensure that the reagents were sufficiently pure to avoid their catalytic decomposition. We used pressure-resistant cells with Suprasil windows⁵. To account for the small difference between the optical paths of the sample and reference cells which could cause some solvent absorption in the ultraviolet region, we systematically recorded the base line with pure ammonia in both cells. To record the spectra for a wide range of alkali metal concentrations, various optical path lengths from 10^{−2} to 10 mm were used.

The temperature was varied by a current of cold nitrogen gas and we checked that thermal equilibrium was reached before recording the spectrum, otherwise the shift of the solvent

Fig. 1 Experimental results for the dependence of the extinction coefficient on wavelength. For potassium-ammonia solutions: ●, −50 °C, 2.35×10^{-3} mol l^{−1}, path length 1.90 mm; ○, 20 °C, 2.03×10^{-3} mol l^{−1}, path length 1.90 mm; ■, −50 °C, 7.3×10^{-4} mol l^{−1}, path length 10.0 mm; □, 20 °C, 6.3×10^{-4} mol l^{−1}, path length 10.0 mm. The results are normalised relative to $\epsilon_{e-am, 700, 20^\circ C} = 1,575$ l mol^{−1} cm^{−1} and $\epsilon_{e-am, 700, -50^\circ C} = 2,600$ l mol^{−1} cm^{−1}. Dashed curves: amide spectra; a, −50 °C; b, 20 °C. $\epsilon_{NH_2^-, 348, 20^\circ C} = 2,600$ l mol^{−1} cm^{−1}; $\epsilon_{NH_2^-, 330, -50^\circ C} = 3,040$ l mol^{−1} cm^{−1}. Results are mean values for the concentration range 5×10^{-4} – 2×10^{-3} mol l^{−1}.



band with temperature results in a pseudo absorption within the studied range. Spectra were recorded with Beckman ACTA IV M and Beckman DK-1A spectrophotometers flushed with nitrogen gas.

At low temperature (-50°C) the rate of decomposition is usually negligible but at room temperature the reaction is catalysed by the walls, especially when using the thin cells for which the surface-to-volume ratio is very high. The absorbances were first followed kinetically for a short time in the infrared band of e_{am}^- , for instance at 700 nm, and at the maximum of the amide absorption, $\lambda = 348\text{ nm}$ (20°C). These measurements revealed the extent to which decomposition occurred during the recording and when necessary allowed the spectra to be corrected.

We examined many more or less stable solutions. The results presented Fig. 1 correspond to the best runs. The optical densities were normalised at 700 nm and the shape of the infrared spectra between 500 and 800 nm at 25°C and between 700 and 1,000 nm at -50°C agree with those found previously^{4,6}.

The spectra normalised relative to A_{700} were plotted assuming an extinction coefficient at 700 nm $\epsilon_{e_{am}^-} = 1,575\text{ l mol}^{-1}\text{ cm}^{-1}$ at 20°C , and $\epsilon_{e_{am}^-} = 2,600\text{ l mol}^{-1}\text{ cm}^{-1}$ at -50°C (refs 4 and 6). It appears (Fig. 1) that the spectrum consists of a second band in the ultraviolet superposed on the tail of the large infrared band and that no characteristic absorption of NH_2^- is present. Only at room temperature and at a potassium concentration of $2 \times 10^{-3}\text{ mol l}^{-1}$ was a weak amide band present, but its contribution was easily subtracted since the amide spectrum is accurately known down to 230 nm (Fig. 1)^{7,8}. Even when the amide absorption increases with time, the kinetics show that the absorption at 348 nm and 230 nm increases with the decay of the infrared band (isobestic point at $402 \pm 2\text{ nm}$). But the increase in the ultraviolet absorption is not at all that expected from the amide spectrum. This means that a part of the ultraviolet absorption should be attributed to e_{am}^- and so the apparent extinction coefficient ($\epsilon_{\text{NH}_2^-} - \epsilon_{e_{am}^-}$)

enables us to determine corresponding $\epsilon_{e_{am}^-}$ values in this region. These are in good agreement with those deduced from direct observation of undecomposed solutions (see below). This method of subtracting the amide spectrum is the only one which can be applied to high concentrations ($\sim 10^{-2}\text{ mol l}^{-1}$), for which the decay is rapid. In this case the same spectrum was obtained, indicating that ultraviolet absorption occurs when the electron is clearly associated with ion aggregates.

We note, for the best runs presented in Fig. 1, that the results agree for both concentrations, that is $6.3 \times 10^{-4}\text{ mol l}^{-1}$ at 20°C (optical path 10 mm) and $2.0 \times 10^{-3}\text{ mol l}^{-1}$ at 20°C (optical path 1.9 mm), showing that the ultraviolet absorption is well correlated with the infrared absorption of e_{am}^- . Comparing the spectra of the same solutions at room and low temperature, Fig. 1 shows that the blueshift of the wide infrared band is still observed in the plateau region (300–400 nm) resulting in values

$$\epsilon_{350, 20^{\circ}\text{C}} = 300 \pm 30\text{ l mol}^{-1}\text{ cm}^{-1}$$

and

$$\epsilon_{350, -50^{\circ}\text{C}} = 390 \pm 30\text{ l mol}^{-1}\text{ cm}^{-1}$$

At lower wavelengths, we observe absorptions merging into the solvent band as in the case of e_{aq}^-

$$\epsilon_{245, 20^{\circ}\text{C}} = 950 \pm 100\text{ l mol}^{-1}\text{ cm}^{-1}$$

and

$$\epsilon_{240, -50^{\circ}\text{C}} = 1,400 \pm 100\text{ l mol}^{-1}\text{ cm}^{-1}$$

The ultraviolet absorption from the solvated electron in liquid ammonia increases at $\lesssim 280\text{ nm}$ while the solvent absorption only starts to rise at $\lesssim 230\text{ nm}$. In water the respective values are 220 nm and 180 nm. Although it may not be

decisive, this correlated displacement supports Hart's interpretation that the redshift of the absorption band of the solvent molecules is caused by perturbations attributable to the electron.

This conclusion is strengthened by the observed temperature effect. Indeed, Fig. 1 shows that the ultraviolet absorption of the ammonia solution starts at higher wavelengths at 20°C than at -50°C as is the case for the band of the pure solvent itself which exhibits a blueshift of $\sim 7\text{ nm}$ when the temperature is lowered over the same range (to be published).

If we admit the above interpretation, the extinction coefficient in the ultraviolet range is not a direct characteristic of the solvated electron but is a parameter determined by the molecules in the solvation shell. In the semi-continuum model^{9–11}, one must take into account interactions which become weaker with successive layers around the electron. The observed ultraviolet absorption is an averaged sum of the perturbations of transitions of these solvent molecules.

A rough approximation which assures the involvement of a limited number of solvent molecules in the solvation sphere of the electron, four for example, leads to extinction coefficients four times smaller than those indicated experimentally. The band obtained is similar to that of the pure solvent and the shift ($\sim 27\text{ nm}$) gives a magnitude of the energy of the perturbation of $\sim 0.6\text{ eV}$.

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Rainfall characteristics in eastern Sahel

THE recent drought conditions in the Sahel region of Africa have generated studies of long term trends in rainfall and monthly or seasonal atmospheric conditions related to the area^{1–3}. The results of these studies, in general, indicate no specific trends in precipitation³, and some evidence of direct¹ and/or long distance² relationships between monthly or seasonal atmospheric state and Sahelian rainfall. We present here the results of a study which suggest the major factor affecting rainfall during the drought was a change in easterly wave activity over northern tropical Africa.

The occurrence and characteristics of easterly waves over northern tropical Africa have been discussed by others^{4–8}. In relation to easterly waves and precipitation, Burpee⁸ has shown that modulation of rainfall in the region between 0 and 18°W is related to wave passage. He found no clear evidence, however, of a similar wave-rainfall relationship between 0 and 15°E . In the Sudan, Hammer⁹ found that spatial and temporal patterns of rainfall approximated the 4–5-d period commonly associated with easterly waves. From evaluation of weather satellite imagery, it has been estimated that 90% of the occurrence of widespread heavy rainfall is directly related to easterly wave passage¹⁰.

To define more clearly the characteristics of Sudanese rainfall, daily cumulative rainfall totals from 200 stations

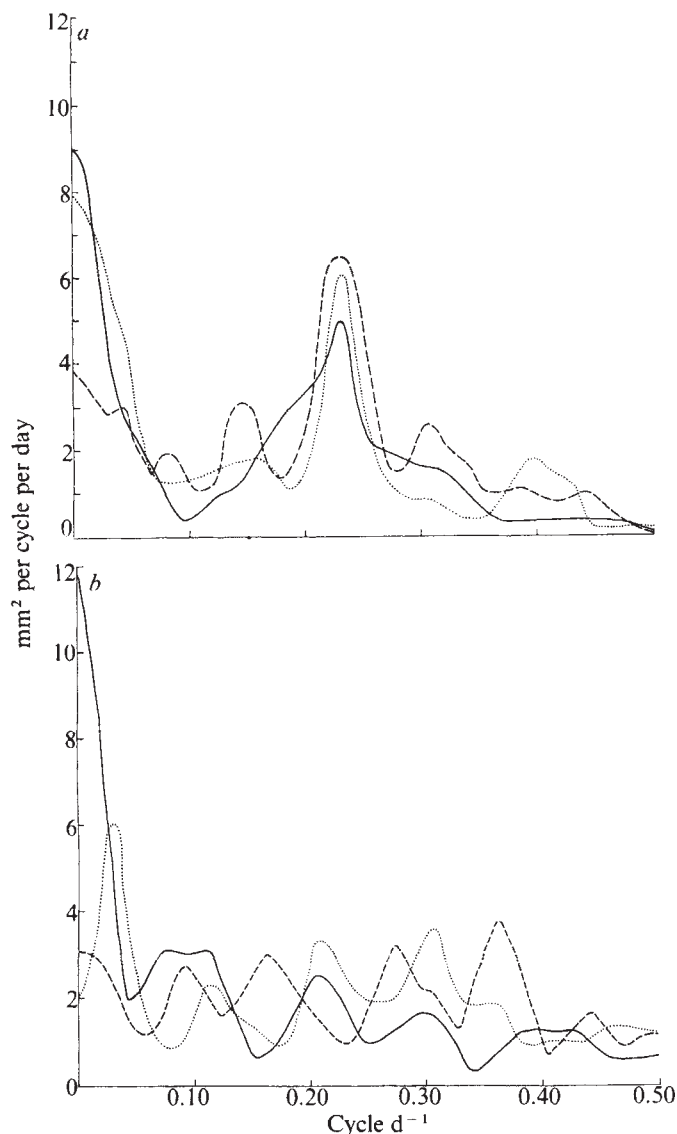


Fig. 1 *a*, Power spectrum analysis results for 90-d period June 13–September 10, 1953 (—), 1961 (·····) and 1969 (---). *b*, As in *a* for 1968 (·····), 1970 (—) and 1971 (---).

in the Sudan were analysed using power spectral techniques. About 75%, a higher proportion in the latter years, were stations located north of 11°N or zonally equivalent to the main drought area of Africa. Unfiltered precipitation totals between June 13 and September 10—the main portion of the rain season—for 1953, 1961, 1968, 1969, 1970 and 1971 were used in the analyses. The spectral analyses were carried out using the OS-3 ARAND system routine SPECT 1 of Oregon State University, and the results are shown in Fig. 1*a* and *b*. The bandwidth used was 0.031 cycle d⁻¹.

Figure 1*a* presents the spectral curves for the years 1953, 1961 and 1969. 1953 and 1961 were years of average to above average rainfall for most of the Sudan during the months of study. The factor accounting for most of the variance in the spectra of 1953 and 1961 was the movement of the so-called Intertropical Convergence (ITC) as shown by the high values at 1 cycle per 90 d (0.011 cycle d⁻¹). For these two years, major variance is also contained within the period 3.7–5 d (0.27–0.20 cycle d⁻¹). This corresponds to the most frequently cited period associated with easterly wave activity⁸. 1969 had a somewhat enigmatic rain season in that in spite of being in the drought sequence, rainfall was near normal in the Sudan. This was similar to precipitation conditions during 1969 in western and central Africa, as found^{3,4,11} from integrated station data. The

small amount of variance contained under the 1 cycle per 90-d portion of the curve for 1969 probably reflects the stagnation of the ITC in July, which limited rainfall over the northern half of the Sudan. Because of the restricted effect of the ITC on the 1969 spectrum, caution must be used in interpreting the variance accounted for under the 3.7–5-d portion of the spectrum as being as relatively significant as it seems compared with 1953 and 1961. A frequency domain test was carried out using the OSU OS-3 ARAND system routine NOIZT to determine if each of the time series could be considered 'white noise'. All five peaks on Fig. 1*a* were significant at the 95% level, indicating they were not the result of a purely random process.

In comparison with the three years already discussed, 1968, 1970 and 1971 display a very different spectral pattern (Fig. 1*b*). Although ITC movement in 1970 was statistically significant (evaluated by the NOIZT routine), the remainder of the spectra for the three years are notably less peaked and temporally coincident than those of Fig. 1*a*, with the exception of a possible 20–40-d period in 1968. Rainfall amounts recorded in the Sudan were markedly below average during 1968, 1969 and 1970. Of the nine months, which comprised most of the 90-d period analysed during these three years, five months had 51–75% of the stations with below average monthly rainfall and two additional months with 46–50% of the stations reporting less than average precipitation. The overall appearance of Fig. 1*b* presents a more random peak occurrence pattern than Fig. 1*a*, particularly in the shorter periods. The rainfall of the three dry years thus seems to be responding to a fluctuating rainfall producing mechanism, in contrast to the relatively constant short period pulses of the more humid years.

Visual evaluation of weather satellite imagery of July and August for 1969, 1970 and 1971 confirms the variability of wave conditions over north-eastern tropical Africa (unpublished). Easterly wave-associated cloud pattern passage intervals were notably different during the three years. 1969 had a reasonably consistent 4–5-d period between wave occurrences; in 1970, most waves were spaced at 2–3 d intervals, with an occasional intervening 5-d period between waves; and 1971 was the most erratic year in terms of the period of the occurrence of the wave. Wave periods ranged from 2–9 d, with 2-d spacing most common. For all three years ~ 65% of the waves were conservative for 1–4 d and could be followed from over the Sudan to central Africa, where the satellite tracking used in this study terminated.

Within the Sudan, the drier rain seasons seem to experience rainfall events in a more spasmodic fashion than wetter rain seasons, and major rainfalls are related to easterly wave passage. In addition, the Sudan lies 'upstream', in terms of easterly wave activity, of the western and central African drought areas and there has been generally synchronous rainfall during recent years between the various regions. Thus, there seems to be a strong probability that during the recent drought the easterly wave operated in an unusual manner, with a consequent reduction in rainfall. The intra- and/or extra-wave factors related to modification of wave conditions over continental Africa are still in question.

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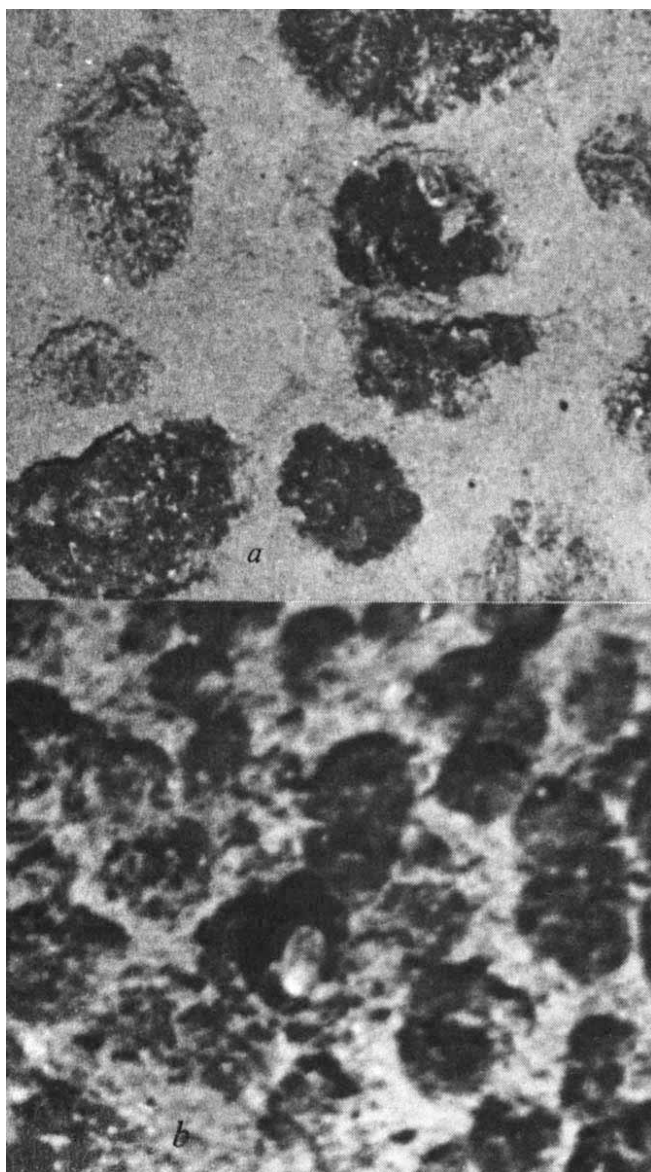
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Deep-sea bottom photographs show that benthic organisms remove sediment cover from manganese nodules

MARINE geologists, geochemists, biologists and other oceanographers have studied manganese nodules since they were first reported by Murray and Renard¹ in 1891. Much has been learned (see ref. 2 for a recent review) but many

Fig. 1 Bottom photographs from the manganese nodule province of the North Equatorial Pacific Ocean. A polyplacophoran (*Chiton*) has removed sediment cover from through nodules (a) and has almost cleaned the nodule it is on (arrowed). Note the sediment covering adjacent nodules. Another *Chiton* (b) is feeding on nodule surface. Average nodule in this area is 6–8 cm in maximum length.

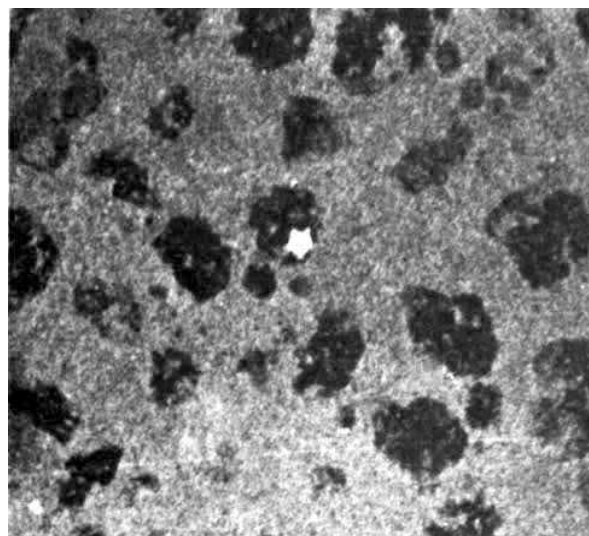


aspects of their formation and growth are open to question. The Environmental Research Laboratory of the National Oceanic and Atmospheric Administration has sponsored a multi-university, multi-disciplinary investigation to determine the environmental impact of manganese nodule mining. As part of the benthic baseline study of the Deep Ocean Mining Environmental Survey (DOMES), approximately 10,000 bottom photographs have been taken. I wish to report a close examination of 365 prints taken on one lowering of the camera near latitude 14°17.5'N, longitude 126°15.4'W at a depth of about 4,500 m.

The following epibenthonts were recognised: 99 cidaroid echinoids; 45 ophiurid ophiuroids; 32 elapod holothurians; 21 actiniarid zoantharia; 8 pennatulid anthozoans; 5 bryozoans; 4 gorgonid anthozoans; 4 asteroids; 3 porifera; 3 lepidopleurid polyplacophora; 2 pycnogonida; 2 brachyuran crabs, and one each crinoid, ascidacean and gastropod. The area of each photograph was calculated using the average size of nodules collected at that station as a scale. The average size was 7.4 cm (± 0.7), and it was determined that 3,031 m² (± 303) were seen, taking into account the few frames where overlapping occurred. Consequently the epifaunal density is 0.08 organisms per m² (± 0.008), which agrees with photographic survey conducted under the oligotrophic Sargasso Sea at similar depths³.

Animal activities such as burrowing under nodules and pushing them upward or ingesting nodules and subsequently egesting them have been suggested⁴ as methods by which the geologically older nodules are kept on top of the younger surficial sediments. These operations may well take place and I would now like to add the mechanism whereby some benthic animals remove sediment from the surface of manganese nodules, apparently as part of their feeding activity. Almost every frame shows evidence of uneven sediment cover on adjacent nodules. In seven frames there are organisms which I believe are grazing and are associated with cleaner nodules (that is, covered with less sediment). The clearest of these photographs are presented here (Figs 1–3). Polyplacophora (chitons) are commonly found in the rocky, intertidal zone where they eat by scraping algae and other material from the rocks⁵. Since manganese nodules are commonly covered with structures of biological origin^{6,7} it is not difficult to hypothesise that chitons in a nodule province feed by removing organic material from nodule surfaces (Fig. 1a and b). Phanerozoid asteroids

Fig. 2 Bottom photograph of phanerozoid asteroid (starfish) feeding on and cleaning manganese nodule surface.



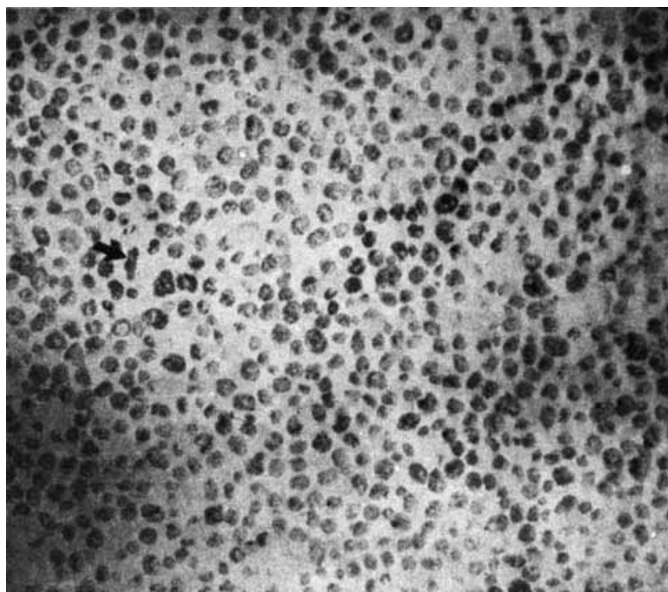


Fig. 3 Bottom photograph of elasipod holothurian (sea cucumber, arrowed). The meandering trail of darker nodules that starts in the upper right corner and ends at the holothurian indicates its path and the removal of sediment cover from nodules as it feeds on deposits.

(Fig. 2) eat various foods* and also seem to scrape and clean nodules. Elasipod holothurians are deposit feeders (Fig. 3) and in addition to possibly nudging nodules and keeping them at the sediment-water interface, they ingest sediment from the nodule surface.

Although the sedimentation rate in this area is low⁸, sediment redistribution is continually taking place. It is not possible to estimate the rate of either cleaning or bottom redistribution of sediment, but it is evident from these photographs that periodic cleaning takes place. If nodules grow on their upper surfaces this episodic cleaning may help to explain their intermittent growth.

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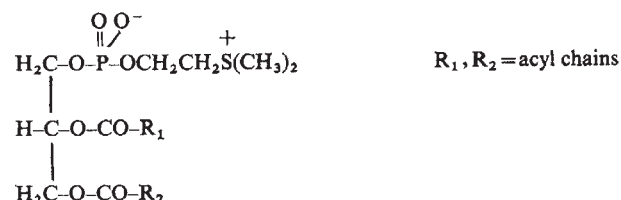
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Sulphonium analogue of lecithin in diatoms

PHOSPHATIDYL choline (lecithin) is the most widespread of the phospholipid membrane components of eukaryotic cells, being present in virtually all those investigated so far. The exceptions noted until recently have been blue-green algae¹, the yeast-like fungus *Pullularia pullulans*² and the phytoflagellate, *Ochromonas danica*³. We report here a sulphonium analogue of phosphatidyl

choline which completely replaces lecithin in the non-photosynthetic diatom, *Nitzschia alba*⁴. The new phospholipid is a phosphatidyl *S,S*-dimethyl mercaptoethanol (phosphatidyl sulphocholine) with the following structure:



The lipid was first observed on thin-layer chromatograms of the total lipids of *N. alba* (grown in synthetic seawater⁴) as a major component with mobilities and staining behaviour⁵ similar to those of lecithin (Fig. 1). But no glycerophosphorylcholine was detected on mild alkaline deacylation of the total lipids, indicating that lecithin was absent in this organism. The new lipid appeared to be a phosphosulpholipid by its labelling with both ³⁵S and ³²P when cells were grown in medium containing ³⁵S-sulphate and ³²P-phosphate.

The phosphosulpholipid was isolated in pure form by column chromatography of the total lipids on Biosil A silicic acid, using increasing concentrations of methanol in chloroform as eluting solvents. The sulpholipid appeared in the chloro-methanol 1:3 eluate and after precipitation from chloroform by addition of acetone, it was chromatographically pure. It accounted for 68% of the total lipid phosphorus and had elemental analyses consistent with those expected for a sulphonium analogue of lecithin. We found C, 64.18%; H, 9.25%; N, 0.00; S, 4.25%; P, 3.85%, atomic ratio P/S, 0.94. The calculated values for C_{41.9}H_{73.8}O₈SP (768.73) are C, 65.47%; H, 9.68%; S, 4.17%; P, 4.02%, P/S, 1.00.

The analysis showed the complete absence of nitrogen and the presence of sulphur and phosphorus in an atomic ratio close to 1:1. Fatty acid analysis by gas-liquid chromatography (GLC) on 10% butanediol succinate polyester at 180 °C revealed the presence of 14:0 (30%), 16:0 (8%), 18:1 (12%), 18:2 (8%), 20:5 (27%) and 22:6 (4%) acids, permitting calculation of the molecular formula given above.

The nuclear magnetic resonance (NMR) spectrum of the

Table 1 Proton NMR assignments for phosphatidyl sulphocholine from *N. alba* in CDCl₃

Proton assignment	Phosphatidyl choline* δ (p.p.m.)	Phosphatidyl sulphocholine δ (p.p.m.)	No. of protons calculated	found
CH=CH	5.38	5.38	7.5	7.7
HC-O-	5.1	5.1	1	0.9
CH ₂ O-CO-R	4.36	4.36	4	4.3
CH ₂ O-P (chlorine)				
CH ₂ O-P (glycerol)	3.9	3.9	2	4.3
CH ₂ S	—	3.9	2	
CH ₂ N	3.8	—	—	—
+ N(CH ₃) ₂	3.37	—	—	—
+ S(CH ₃) ₂	—	3.17	6	5.7
=C-CH ₂ -C=	2.83	2.83	5.5	6.0
CH ₂ CO	2.28	2.28	4	4.6
CH ₂ C=	2.08	2.08	4.1	4.5
CH ₂	1.28	1.28	24	22
CH ₃	0.9	0.9	6	6.0†

*Purified egg lecithin (Serdary Research Laboratories). For assignments see ref. 7.

†Taken as reference for integration.

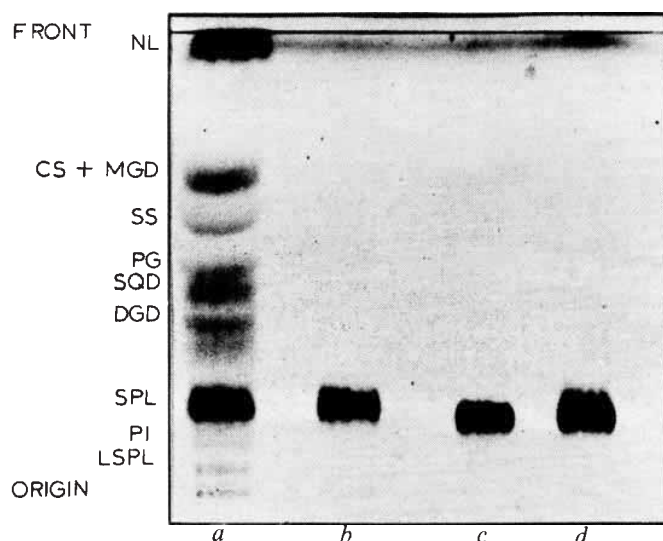


Fig. 1 Thin-layer chromatogram on silica gel H in the solvent system chloroform-methanol-28% ammonia (65:35:5, v/v) of: *a*, total lipids of *N. alba*; *b*, isolated phosphatidyl sulphocholine from *N. alba*; *c*, commercial egg lecithin (PC), and *d*, mixture of phosphatidyl sulphocholine and lecithin. Chromatogram was charred with 50% H_2SO_4 . Phospholipids were detected by the phosphate stain⁵ and "choline"-containing lipids by the Dragendorff reagent⁶. NL, Neutral lipid; CS, ceramide sulphate⁸; MGD, monoglycosyl diglyceride; SS, sterol sulphate⁸; PG, phosphatidyl glycerol; SQD, sulfoquinovosyl diglyceride; DGD, diglycosyl diglyceride; SPL, phosphatidyl sulphocholine; PI, phosphatidyl inositol; LSPL, lysophosphatidyl sulphocholine. SPL and LSPL as well as lecithin gave positive tests with the phosphate and "choline" stains.

sulpholipid showed a sharp signal at 3.17 δ p.p.m. which was assigned to the six protons of the dimethyl sulphonium group. For comparison, the quaternary ammonium group methyl protons of phosphatidyl choline gave a singlet at 3.37 δ p.p.m. (Table 1; ref. 7). The remainder of the spectrum was essentially the same as that of phosphatidyl choline. Assignments of proton signals and their integration (Table 1) are consistent with the structure of phosphatidyl *S,S*-dimethyl mercaptoethanol. The infrared spectrum of the sulpholipid showed peaks at 2,868 and 2,968 cm^{-1} (CH_2 and CH_3), 1,747 cm^{-1} (ester C=O), 1,475 cm^{-1} (CH_2 and CH_3), 1,285 cm^{-1} (P=O), 1,175 cm^{-1} (ester C-O) and a doublet with a major peak at 1,092 cm^{-1} (PO⁻) and a shoulder at 1,065 cm^{-1} (P-O-C). There was no evidence of sulphate (1,440–1,350, 1,230–1,150 cm^{-1}) or sulphonate (1,420–1,330, 1,200–1,145 cm^{-1}) bands. Comparison with the spectrum of phosphatidyl choline revealed

close similarity except for the sharp peak at 970 cm^{-1} which was absent from the spectrum of the sulpholipid and which is generally assigned to the $(CH_3)_3N^+$ group of lecithin^{8,9}.

Enzymatic hydrolysis of the sulpholipid with cabbage phospholipase D yielded: (1) a chloroform-soluble phospholipid identified as phosphatidic acid by cochromatography on silica gel H with an authentic standard (R_f , 0.05 and 0.9 in chloroform-methanol-28% ammonia (65:35:5, v/v) and chloroform-methanol-90% acetic acid (30:4:20, v/v), respectively); and (2) a water-soluble sulphur-containing compound which gave a positive Dragendorff reaction⁵ and had mobilities on paper chromatography and paper electrophoresis (Table 2) identical to those of synthetic 2-hydroxyethyl dimethylsulphonium iodide (*S,S*-dimethyl mercaptoethanol, "sulphocholine", made by methylation of mercaptoethanol with methyl iodide). The latter product was also present in the 2.5% methanolic-HCl hydrolysate of the sulpholipid along with fatty acid methyl esters and two water-soluble periodate-Schiff-positive products identified chromatographically as α -glycerophosphate and methyl- α -glycerophosphate. Identification of the sulphur-containing acid-hydrolysis product as "sulphocholine" was confirmed by monodemethylation of the sulphonium group with sodium benzenethiolate¹⁰. The reaction products had GLC retention times relative to dimethylsulphide (4.59 and 6.04) identical to those of authentic hydroxyethylmethyl sulphide and phenylmethyl sulphide, respectively, on a column of 10% butanedisuccinate polyester on Gaschrom W at 128 °C and a carrier inlet pressure of 0.4 kg cm^{-2} . An identical gas chromatographic tracing was obtained after monodemethylation of synthetic "sulphocholine".

Hydrolysis of the sulpholipid with phospholipase C from *Clostridium perfringens* released: (1) a chloroform-soluble product with R_f value identical to that of standard 1,2-diglyceride (R_f , 0.22 on silica gel H in petroleum ether-diethyl ether-acetic acid 70:30:1, v/v); and (2) a water-soluble phosphorus and sulphur-containing compound with mobilities similar to those of phosphorylcholine (Table 2). Unlike phosphorylcholine, however, the water-soluble product was found to undergo methanolysis in methanolic 0.2 N NaOH at 37 °C for 1 h, with quantitative release of inorganic phosphate and formation of methoxysulphocholine (2-methoxyethyl dimethylsulphonium salt) identified chromatographically and electrophoretically in comparison with an authentic standard (Table 2). The water-soluble product formed by phospholipase C hydrolysis is thus probably phosphorylsulphocholine.

Mild alkaline deacylation of the phosphatidyl sulphocholine in methanolic NaOH also yielded methoxysulphocholine, together with α -glycerophosphate (Table 2) instead of the expected glycerophosphoryl sulphocholine. Apparently the phosphate-sulphocholine linkage both in phosphatidyl sulpho-

Table 2 R_f values and electrophoretic mobilities of the sulphur-containing hydrolysis products from phosphatidyl sulphocholine

Hydrolytic procedure	R_f in butanol-propionic acid-water (142:71:100, v/v)	Relative electrophoretic mobility towards cathode*	
		pH 2.1	pH 6.5
Methanolic HCl†	0.52	1.00	1.00
Mild alkali‡	0.72	1.00	1.00
Phospholipase D	0.52	1.00	1.00
Phospholipase C	0.23	0.00	-0.05
Standards			
Choline	0.57	1.00	1.00
Phosphorylcholine	0.23	0.00	-0.05
2-Hydroxyethyl dimethylsulphonium iodide	0.52	1.00	1.00
2-Methoxyethyl dimethylsulphonium iodide	0.72	0.98	0.97
Trimethylsulphonium iodide	0.54	1.17	1.20
Butyldimethylsulphonium iodide	0.80	0.90	0.89

*Relative to mobility of choline.

†Hydrolysed in 2.5% methanolic HCl for 5 h under reflux; water-soluble phosphates detected were glycerophosphate and methylglycerophosphate (R_f in butanol-propionic acid-water 142:71:100, v/v: 0.17 and 0.30, respectively).

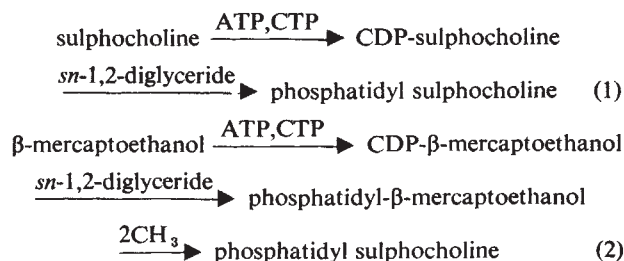
‡According to procedure of Kates⁵, neutralised with ethyl formate; the water-soluble phosphate detected was α -glycerophosphate (R_f 0.17 and 0.31 in butanol-propionic acid-water 142:71:100 (v/v) and phenol-water 100:38 (w/w) respectively).

choline and in phosphorylsulphocholine is readily attacked by methoxide ion, resulting in the release of the methoxysulphocholine with concomitant formation of α -glycerophosphate or inorganic phosphate, respectively.

The anomalous behaviour of phosphatidyl sulphocholine towards mild alkali may explain the unusual phospholipid composition of several photosynthetic marine diatoms reported previously¹¹. Low amounts of glycerophosphorylcholine (3–8%) and high amounts of α -glycerophosphate (11–32%), as well as a ³⁵S-labelled compound (ref. 11, Fig. 8, spot 11) with mobilities similar to those of methoxysulphocholine, were detected in mild alkaline hydrolysates of the total lipids of these diatoms. Also, a ³²P-labelled and ³⁵S-labelled spot with the mobilities of lecithin was detected on chromatograms of the intact lipids of these organisms, indicating the presence of phosphatidyl sulphocholine (ref. 11, Fig. 1 and 7).

From these observations and the findings reported here, it seems that phosphatidyl sulphocholine may be present in high proportions in marine diatoms, replacing phosphatidyl choline as the major membrane phospholipid component. Phosphatidyl sulphocholine would be expected to have a zwitterionic structure at pH 7 and overall size and shape similar to lecithin so that this novel lipid could functionally substitute for lecithin in membranes of *N. alba* and other diatoms.

The question concerning the biosynthetic pathways for the sulphonium analogue is intriguing, and two possible pathways can be postulated by analogy with those for phosphatidyl choline in mammalian and plant systems¹²



In pathway (1), sulphocholine might be derived from dimethyl β -propiethetin, which is commonly found in marine organisms^{13–15} and has been shown to be derived from methionine¹⁴. Alternatively, the sulphocholine might arise from methylation of β -mercaptoethanol, which is known to be a substrate for methyltransferases in rat liver¹⁶. Evidence for the operation of pathway (1) has been reported for a mammalian system by Bjerre and Bremer¹⁷ who found that radioactive label from both ³⁵S-sulphocholine and Me-³H-sulphocholine was incorporated in rat heart and kidney phospholipids. With regard to pathway (2), we have not detected the presence of the expected phosphatidyl mercaptoethanol in *N. alba*, but this would not necessarily eliminate pathway (2) because rapid turnover of the thiol intermediate would make its detection difficult.

The absence in *N. alba* and other marine diatoms¹¹ of significant amounts of nitrogenous phospholipids (phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine) and the high concentrations of phosphatidyl sulphocholine and ceramide sulphonate⁶ suggest that in these organisms sulphur-containing amino acids (for example, cysteine and methionine) rather than serine, probably act as primary metabolic precursors of the glycerophospholipids and sphingolipids. These novel biosynthetic pathways are being investigated.

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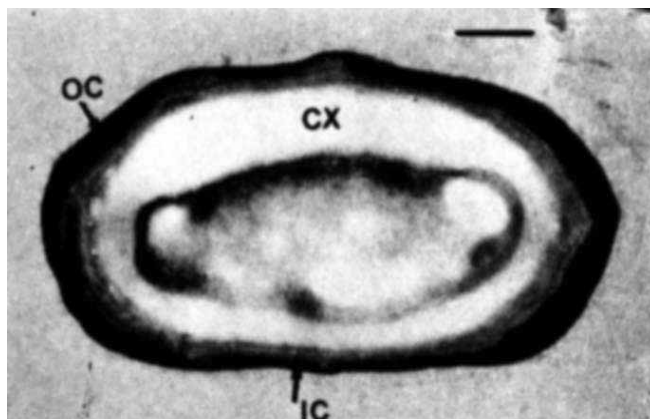
An exosporium-like outer layer in *Bacillus subtilis* spores

BACTERIAL endospores are differentiated cells surrounded by several characteristic spore envelopes—cortex, inner and outer spore coats. In addition to these layers, present in all *Bacillus* and *Clostridium* spores, several species possess a loosely fitting outermost structure—the exosporium^{1–5}. In other species, however, including *B. subtilis*, the exosporium has not been found in thin sections^{1,6,7}, although its possible existence was suggested by the observation of a fibrous ‘exosporial’ layer by freeze-etching⁸. In our studies of the fine structure of *B. subtilis* spores we have found that a new outer layer, probably an exosporium, is present and can be visualised in thin sections after partial chemical extraction of the spore coats.

PM9, a normally sporulating, auxotrophic (Trp C2, Met C3, Phe A1) strain derived from *B. subtilis* Marburg 168 was used. Spores were obtained in a complex nutrient broth⁹ and fixed for electron microscopy either untreated or after incubation for 2 h at 37 °C in 0.1 M Tris, 8 M urea, at pH 8.5 in the presence of 1% mercaptoethanol^{10,11}. Fixation, staining and electron microscopy were carried out as described previously¹².

Untreated spores show the classical spore structure with a cortex, an inner coat formed by three well defined layers and a thick, darker outer coat (Fig. 1). After urea–mercaptoethanol treatment (Fig. 2), the cortex and the inner coat remain unchanged, but the outer coat appears more loose and granular. In addition, a loosely fitting single sheet, detached from the

Fig. 1 Untreated free mature spore of *B. subtilis* PM9 exhibiting the typical fine structure of spore envelopes. CX, Spore cortex; IC, inner spore coat; OC, outer spore coat. Note that no exosporium is clearly visible. Bar represents 0.2 μ m.



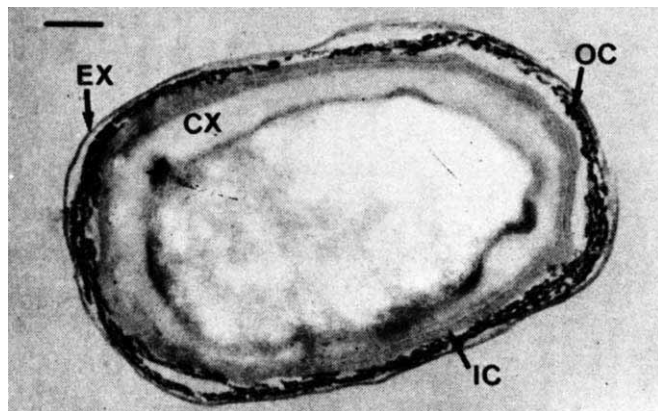


Fig. 2 Free mature spore of *B. subtilis* PM9, treated with urea and mercaptoethanol. The three envelopes visible in the control (Fig. 1) are present. The fine structure of the outer spore coat is disorganised. Note the clearly defined outermost layer which exhibits all the characteristics of an exosporium (EX). Bar represents 0.2 μ m.

outer coat, is visible. This layer is very much like the well known exosporium of *B. cereus* and other species³⁻⁵.

Treatment of spores with a reducing agent in the presence of urea is known to liberate a large amount of insoluble, structural proteins from the spore coats^{11,12}, as reflected in the morphological changes of the coat structure. Our observations suggest the existence of a new, exosporium-like layer, tightly fitting the spore and masked by the dense outer spore coat. In fact, the exosporium is clearly visible in occasional sections of untreated spores, in which it appears tightly fitting the outer surface and remains in most cases undistinguishable from the dark outer coat. The clarity with which the exosporium could be seen, seems to be the result of two effects of the urea-mercaptoethanol treatment—partial dissolution of the outer coat and loosening of the exosporium.

Similar results were obtained with other wild-type and mutant *B. subtilis* strains. Control experiments show that the treatment which enables visualisation of 'exosporium' does not destroy the viability and heat resistance of the spores. It has been suggested¹³ that the exosporium may play a role in the resistance of the spore—that is, against proteases and lytic enzymes. Its presence in *B. subtilis* spores would explain why there is no marked difference between the properties of these spores and those of such species as *B. cereus*.

Demonstration of an exosporium-like structure in *B. subtilis* implies that the classification of bacterial spores into two classes¹², with and without exosporium, is incorrect, and extends the morphological similarity of the spores of various species to the outermost layer.

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Bacteriophage P22 lysogenises efficiently at high multiplicities of infection because *Salmonella typhimurium* DNA synthetic capacity is limited

INFECTIONS of *Salmonella typhimurium* by wild-type phage P22 generally result in a lytic response at low multiplicities of infection (MOIs) and a lysogenic response at high MOIs¹. At MOIs above 5, more than 90% of all cells are lysogenised. At an MOI of 3, 45% of the infected cells become lysogens whereas at an MOI of 1, only 23% of the infected cells are lysogenised. The regulation of P22 lysogeny has been studied extensively¹⁻⁷. Three structural genes and a *cis*-active site in the immunity C (*immC*) region are required to establish lysogeny. Interaction of gene *c1* and *c3* products at the *c27* site stimulates the production of the *c2* repressor, and at the same time causes a transient retardation in the expression of lytic genes^{1,8}. A mutant of *S. typhimurium*, Pox-1, that channels P22 infections at any MOI to lysogeny with an efficiency of 1.0 has a reduced capacity for both host and viral DNA synthesis⁹. Slowed synthesis of DNA in this mutant results in an overproduction of regulatory proteins relative to the levels of viral DNA in infected cells. This, in turn, results in lysogeny at all MOIs. We now propose that the initial decision

Fig. 1 Effects of MOI on rates of incorporation of ³H-thymidine. *S. typhimurium* strain 109 was infected at 30 °C with wild-type P22 at the indicated MOIs. Samples (10⁸ cells) were pulse labelled with 20 μ Ci of ³H-thymidine⁹. ○, Uninfected control; ×, MOI 3; △, MOI 5; ▲, MOI 10. The dotted line is an arbitrary base line indicating possible levels of host DNA synthesis (see text). Dashed arrows mark the areas between 6 and 15 min after infection.

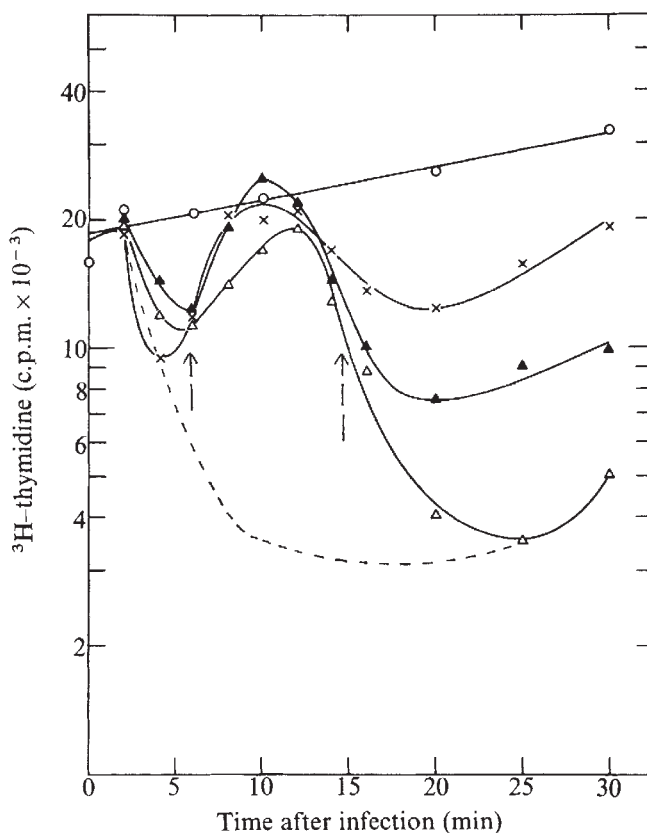


Table 1 Percentage hybridisation of DNA and RNA from infected cells to P22 DNA

	1	2	3	10	20
(a) DNA-DNA hybridisation	24%	19%	21%	44%	32%
(b) RNA-DNA hybridisation	—	—	1.8%	6.3%	10.3%

a, Hybridisation of ^3H -thymidine-labelled DNA from infected cells to P22 DNA. ^3H -thymidine ($200\ \mu\text{Ci ml}^{-1}$) was added to strain 109 6 min after infection and the cells were lysed 20 min after infection as described before¹¹. Hybridisation to P22 DNA was done by the method of Denhardt¹². Data are expressed as the percentage of input counts bound to P22 DNA. Values for MOIs of 1 and 2 were corrected for the presence of uninfected cells in those cultures. b, Hybridisation of ^3H -uridine-labelled RNA from infected cells to P22 DNA. RNA was pulse labelled with ^3H -uridine ($15\ \text{Ci ml}^{-1}$) for 1 min, 8 min after infection of strain 109 by wild-type P22. RNA was prepared and hybridised to P22 DNA by the method of Smith¹³.

between lysis and lysogeny in wild-type cells is also a function of the relative amounts of phage DNA and the phage proteins from the *immC* region.

Wild-type cells were infected with wild-type P22 at MOIs of 3, 5, and 10, and the rates of ^3H -thymidine incorporation were determined (Fig. 1). The initial decline in incorporation rate during the first 6 min was a result of shutoff of host DNA synthesis. The extent of host shutoff was comparable in the three infections. Phage DNA synthesis is required for both lysis and lysogeny. The areas under the curves between 6 and 15 min represent such synthesis⁴. Incorporation of ^3H -thymidine between 15 and 30 min at MOIs 3 and 5 was primarily into cells which were lytically infected. An arbitrary base line of host DNA synthesis rate is drawn at $3 \times 10^3\ \text{c.p.m. min}^{-1}$. This is the level seen by Levine and Schott⁴, using a phage mutant unable to synthesise DNA. The areas under the curves between 6 and 15 min were integrated to estimate the relative amounts of viral DNA synthesised in the three infections. Total calculated c.p.m. are 9.1×10^4 , 7.1×10^4 , and 9.0×10^4 at MOIs of 3, 5 and 10, respectively. The ratios of total c.p.m. incorporated ($1.29:1.0:1.28$) are different from the ratios of the parental genomes available for replication. These ratios, and the fact that the heights of the three peaks are comparable, suggest that the maximum replication of parental genomes was reached at an MOI of 3 or less.

Table 1a shows the results of hybridising DNA from infected cells to P22 DNA attached to nitrocellulose filters. The ratio of percentage hybridisation for MOI 1 to that for MOI 20 is $1.0:1.33$, and is much different from the ratio of the parental genomes. It is reasonable to suggest that near maximum replication of P22 DNA occurs at an MOI of 1. Moreover, these data support our conclusion that host shutoff does not depend on MOI, for the fraction of DNA that is virus specific is nearly constant.

RNA synthesis did not follow the same pattern as DNA synthesis. Figure 2 shows the incorporation of ^3H -uridine pulses into acid-precipitable material. There were almost fourfold differences in both initial decreases and in maximum levels of incorporation seen at MOIs of 3 and 20. Differences in the degree to which host specific transcription was shutoff probably accounts for the variation in incorporation during the first 2–4 min. This makes interpretation of the curves difficult, since each curve represents the sum of host and phage-specific RNA, and the amount of host RNA may be different in each infection.

We determined the amount of viral-specific RNA synthesised 8 min after infection by hybridising RNA from infected cells to P22 DNA. The results are shown in Table 1b. As MOI increased, the total incorporation of ^3H -uridine decreased (Fig. 2), but the fraction that was virus specific increased. Two conclusions are drawn from these data. First, the synthesis of phage RNA was directly dependent

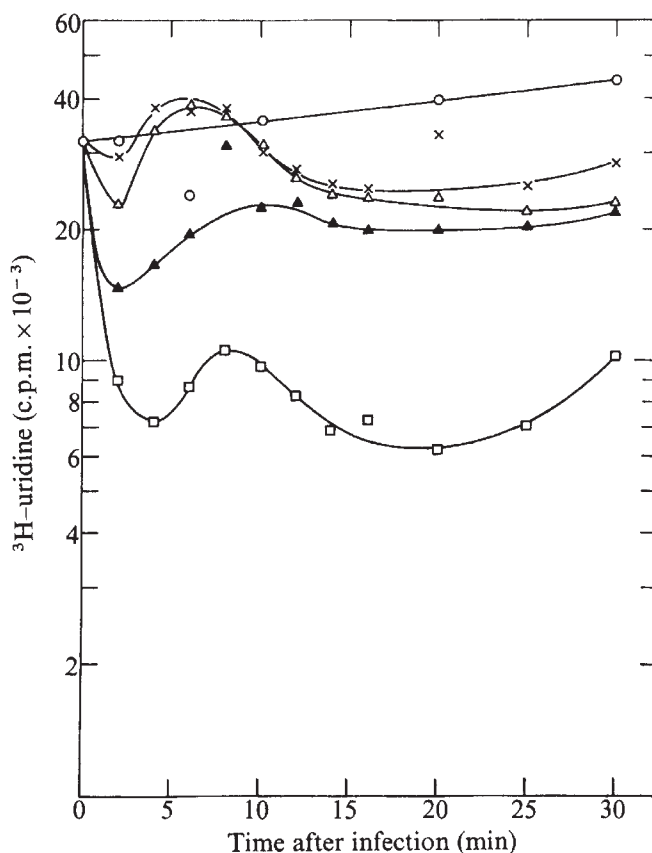


Fig. 2 Effects of MOI on rates of incorporation of ^3H -uridine. Strain 109 was pulse labelled with $40\ \mu\text{Ci}$ of ^3H -uridine using the same procedure as for ^3H -thymidine incorporation⁴. $\circ$, Uninfected control; $\times$, MOI 3; $\triangle$, MOI 5; $\blacktriangle$, MOI 10; $\square$, MOI 20.

on MOI. The ratio of the percentage hybridisable counts at MOI 3 to percentage hybridisable counts at MOI 20 is $1.0:5.5$, close to the ratio of MOI 3 to MOI 20 which is $1.0:6.6$. Second, because the fraction of RNA that was virus specific increased with MOI, the fraction that was host specific must have decreased. Therefore, the extent of host shutoff after infection was also MOI dependent.

There is no evidence for translational controls in P22 infections. Therefore, increased levels of RNA synthesis at high MOIs should result in increased levels of protein synthesis. We expect the *c1* gene transcript to be among the few species of phage RNA that are made 8 min after infection¹. The *c1* protein is pivotal in the establishment of lysogeny^{1-3,5-8}. If higher MOIs result in enhanced transcription of this gene, then the ratio of *c1* protein to DNA would be increased at higher MOIs. At present, there is no assay for the *c1* protein. Therefore we are restricted to measurements of RNA.

If cells are infected with wild-type P22 at various MOIs, and the percentage of infected cells receiving 3 or more copies of the parental genome is calculated from a Poisson distribution, there is good agreement between these values and the percentage of infected cells that become lysogens. We suggest that in a cell infected with 1 or 2 viral genomes, the rate of DNA synthesis relative to RNA synthesis is sufficiently rapid that not all the viral DNA can be complexed with *c1* protein, and the infection proceeds to lysis. If three or more copies of phage DNA enter a host cell, the rate of production of *c1* protein is sufficient to bind all the DNA and the cell is lysogenised. The dependence of lysogeny on MOI is explained by the limited capacity of the infected cell to make DNA; this is saturated at MOIs near 1. RNA synthesis is not so limited. Therefore, at high MOI,

the ratio of RNA synthesis (and regulatory proteins) to DNA synthesis is enhanced. When the ratio of regulatory proteins to DNA is sufficiently high, infections go to lysogeny. This is achieved at MOIs of 3 and more in P22.

Phage P22 has been classified as a lambdoid phage⁹. Phage λ also shows increased frequencies of lysogeny at higher MOI¹⁰. We expect that limited DNA synthetic capacity of the infected bacteria accounts for the increased frequencies.

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Acquired resistance to infection with *Schistosoma mansoni* induced by *Toxoplasma gondii*

SPECIFIC acquired immunity to *Schistosoma mansoni* in the mouse has only recently been demonstrated definitively¹. This phenomenon does not occur until 12 weeks after primary infection and has been found to be adoptively transferred only by serum². The induction of so-called 'immunity' to *S. mansoni* has also been reported in mice and hamsters exposed to a wide variety of unrelated organisms and extracts^{3–6}, but in most, if not all cases it is likely to be nonspecific. Although all of these acquired forms of resistance have been lumped together under the name of immunity, it is probable that the mechanisms of specific and nonspecific resistance are totally different. The present investigation of the effect on *S. mansoni* infection of *Toxoplasma gondii*, an intracellular protozoan parasite which renders mice resistant to a variety of unrelated organisms^{7–11}, largely by activation of macrophages¹², may clarify the role of nonspecific mechanisms in protection against schistosomiasis.

Swiss albino female mice (18–20 g) were purchased from

Carworth Farms, New City, New York, although in one experiment mice were obtained from Flow Laboratories, Rockville, Maryland. *T. gondii* cysts were obtained from the brains of animals infected for 5–6 weeks with the Gleadle strain. Experimental animals were injected intraperitoneally with 10 cysts suspended in 0.2 ml brain emulsion to which penicillin (100 U ml⁻¹) and streptomycin (10 μ g ml⁻¹) were added. Similar volumes of brain emulsion from uninfected mice containing antibiotics were injected intraperitoneally into control animals.

Recovery of immature schistosomes (schistosomula) from the lungs was carried out in two separate experiments in groups of eight *Toxoplasma*-infected mice and eight control animals (injected with normal mouse brain emulsion) 2 weeks after the initiation of the protozoan infection. The animals were anaesthetised and exposed percutaneously to 500 cercariae of a Puerto Rican strain of *S. mansoni*¹³. Recovery of schistosomula was carried out on day 6 after infection (the day of maximum recovery for the strain of mice used) as described by Sher *et al.*¹.

For quantitation of adult worms, mice were injected subcutaneously with 20 cercariae of a Puerto Rican strain of *S. mansoni*. Six to eight weeks after infection, groups of 10 animals were anaesthetised and the portomesenteric venous system perfused¹⁴. In addition, the livers and mesenteric vessels were searched for worms. All worms recovered from each experimental group were washed in normal saline, counted, and the length of the paired worms measured by an ocular micrometer mounted on a dissecting microscope.

In one experiment the worms were washed three times in normal saline, emulsified in a tissue grinder and injected intraperitoneally into four mice to determine whether or not the schistosomes harboured any *T. gondii*. The animals were killed 6 weeks later, their brains examined for cysts, and their sera tested for fluorescent antibodies¹⁵.

Schistosome eggs were counted by digesting the livers removed from *Toxoplasma*-infected and control mice 8 weeks after infection with *S. mansoni*¹⁶.

In two experiments using mice, respectively, from Carworth Farms and Flow Laboratories, the *Toxoplasma*-infected animals had 35% (61 ± 5 compared with 94 ± 8 in the controls) and 40% (89 ± 10 compared with 148 ± 12 in the controls) reductions in the number of schistosomula recovered 6 d after exposure to *S. mansoni* cercariae. The differences between the experimental and control animals in each case were highly significant ($P < 0.01$).

Table 1 summarises the results of adult worm perfusions 6–8 weeks after *S. mansoni* infection. In two separate experiments, animals infected with *T. gondii* 1 d before exposure to *S. mansoni* developed 32% and 22% fewer total worms, and in each case 50% fewer worm pairs in comparison with controls. The differences in each experiment were significant at the 5% level. Furthermore, measurement

Table 1 Effect of injection of ten *T. gondii* cysts at different times before and after exposure to 20 *S. mansoni* cercariae: numbers and sizes of the adult worms 8 weeks after induction of schistosomiasis

	Males	Mean number of worms $\pm$ s.e.			Mean length of worms (mm $\pm$ s.e.)	
		Females	Pairs	Total	Males	Females
<i>T. gondii</i> 4 weeks before:						
Controls	0.8 ± 0.2	1.0 ± 0.4	3.4 ± 0.5	8.5 ± 0.8	9.2 ± 0.4	12.1 ± 0.4
<i>T. gondii</i> -infected	1.1 ± 0.4	0.6 ± 0.4	2.0 ± 0.4	5.5 ± 0.7	9.0 ± 0.2	11.8 ± 0.6
<i>T. gondii</i> 1 d before:						
Experiment 1						
Controls	3.0 ± 0.5	1.4 ± 0.5	3.2 ± 0.4	10.8 ± 1.2	8.9 ± 0.2	10.8 ± 0.3
<i>T. gondii</i> -infected	2.9 ± 0.5	0.9 ± 0.4	1.6 ± 0.3	7.3 ± 0.8	8.4 ± 0.2	8.8 ± 0.4
Experiment 2						
Controls	2.5 ± 0.6	1.0 ± 0.3	2.4 ± 0.6	9.3 ± 1.1	8.0 ± 0.4	9.6 ± 0.4
<i>T. gondii</i> -infected	3.0 ± 0.5	1.1 ± 0.3	1.2 ± 0.2	6.5 ± 0.4	6.5 ± 0.2	7.4 ± 0.2
<i>T. gondii</i> 4 weeks after:						
Controls	1.1 ± 0.3	0.2 ± 0.1	3.3 ± 0.2	7.9 ± 0.4	8.8 ± 0.4	11.1 ± 0.5
<i>T. gondii</i> -infected	1.1 ± 0.4	0	3.4 ± 0.3	8.5 ± 0.4	9.0 ± 0.5	10.9 ± 0.6

of the paired worms in both experiments showed that in each case the female worms from the *Toxoplasma*-infected mice were significantly shorter ($P < 0.05$) (Table 1). The effect of the time of induction of toxoplasmosis on the recovery of adult schistosomes was then studied. *Toxoplasma* cysts were injected 4 weeks before and 4 weeks after the initiation of the schistosome infection (Table 1). Whereas previous toxoplasmosis resulted in a 35% reduction in the total worm load, infection 4 weeks after the injection of *S. mansoni* cercariae had no effect.

There was no evidence that the adult schistosomes were infected with *T. gondii* as the four mice injected 6 weeks previously with emulsified worms from *Toxoplasma*-infected animals had no cysts in their brains and no antibodies in their sera.

The results of *S. mansoni* egg counts in the livers of the different experimental groups were as follows: control animals injected with normal brain emulsion developed a mean egg load per liver of $6,736 \pm 325$, whereas animals with *T. gondii* infection induced 4 weeks or 1 d before schistosomiasis had respective reductions in the egg counts of 35% ($4,480 \pm 210$) and 43% ($3,840 \pm 301$). In contrast, animals infected with *T. gondii* 4 weeks after *S. mansoni* had mean egg counts similar to the controls ($6,077 \pm 286$).

Toxoplasmosis has been reported to protect mice against a variety of unrelated organisms and tumours⁷⁻¹¹. This effect has been ascribed largely to activation of the macrophages¹². Ruskin *et al.*¹² have demonstrated that activated macrophages from *T. gondii*-infected mice not only display activity *in vivo* but have the capacity *in vitro* to destroy nonspecific target cells by a mechanism which does not involve phagocytosis. It has also been shown that other intracellular infectious agents such as *Besnoitia jellisoni* confer similar properties on mouse peritoneal macrophages¹³.

Schistosomiasis is one of the major helminth infections of mankind, and greater understanding of the host defence mechanisms is urgently needed. The variety of conditions discussed under the blanket heading of immunity, however, tends to be confusing. Only recently has it been demonstrated definitively that specific acquired resistance to reinfection occurs in mice previously infected with *S. mansoni*. This does not occur until 12 weeks after infection and has been transferred passively with serum but not with cells^{1,2}. Using monospecific antieosinophil serum¹⁷ it has been shown that this form of resistance is due to an antibody-dependent cell-mediated mechanism in which the effector cell is the eosinophil¹⁸.

Also considered under the term immunity have been various forms of resistance induced by snail haemolymph³, monkey erythrocytes⁴, formalin-killed *Escherichia coli*⁵ and extracts of *Fasciola hepatica*⁶. It seems probable, however, that most, if not all of these materials induced a nonspecific form of acquired resistance involving mechanisms totally different from that of specific immunity. The present experiments in which *T. gondii* induced resistance in mice to *S. mansoni* strongly suggest that nonspecific resistance can be acquired in schistosomiasis.

Although *T. gondii* activates macrophages¹², a factor considered to be largely responsible for the nonspecific protection it affords against other organisms, this protozoan is also a powerful suppressant of B and T lymphocytes^{14,20}, cells which are necessary for the induction and maintenance of specific immunity. Furthermore, specific acquired resistance in the mouse takes about 3 months to develop, but the protective effects of *T. gondii* and the other nonspecific agents mentioned above are fully manifest within a few days.

With respect to our knowledge of the immunology of schistosomiasis, therefore, it is important not to lump all forms of acquired resistance to infection under the general term immunity, but to determine whether they are specific

or nonspecific. Since both types provide only partial resistance, and their kinetics and mechanisms are different, perhaps their effects may be additive.

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Malaria transmission blocked by immunisation with gametes of the malaria parasite

IN view of the limited success of malaria eradication schemes based on concepts of vector control and chemotherapy, serious efforts are being made to investigate the possibility of developing an antimalarial vaccine. So far two classes of vaccine have been under investigation: (1) an anti-sporozoite vaccine designed to prevent malarial infection by blocking the infectivity of sporozoites introduced by mosquito bite^{1,2}, and (2) vaccines directed against the asexual stages in the blood³⁻⁵. Although the potential value of such vaccines is unquestionable, their realisation faces technical problems⁶. The latest proposition is to induce immunity against the parasite stages which infect the mosquito and by so doing prevent transmission of the disease by the vector. Gwadz has shown that the infectivity of malarious chickens to mosquitoes can be reduced greatly by prior vaccination with formalin-treated or X-irradiated blood infected with the malaria parasite *P. gallinaceum*⁷. The number of oocysts (the stage of the parasite developing on the wall of the mosquito gut) in mosquitoes fed on vaccinated chickens was reduced by 95-98% below that recorded in mosquitoes fed unvaccinated birds. To achieve this reduction, chickens were given three weekly intravenous inoculations of a total of 4.5 ml of formalin-treated or irradiated blood. Using the same schedule, but with partially purified gametes of the malaria parasite, we have now reduced the infectivity of malarious chickens to mosquitoes at least 99.9% below control levels. To achieve

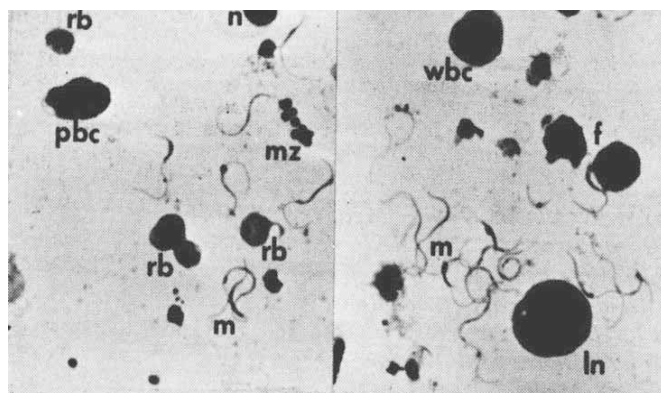


Fig. 1 The material in the 18,000g pellet (see text) used for vaccinating chickens. The preparation has been fixed and stained with Geimsa for the purposes of illustration. m, Male gamete; f, female gamete; rb, residual body of male gametocyte (precursor cell of male gametes) after release of gametes; pbc, parasitised red blood cell; mz, merozoites (mature asexual cells ready to invade red blood cells); n, nucleus of red blood cell within red cell ghost membrane; ln, lysed nucleus of red blood cell; wbc, white blood cell.

this reduction we had to use a minimum of 0.1 μ l of packed cellular material.

We used free gametes for the following reasons. To establish a malarial infection in the mosquito after a blood meal, male and female gametes of the parasite must undergo fertilisation in the lumen of the mosquito midgut. We predicted that this usually rapid process might be blocked by antibodies directed against one or both types of gamete. In the ordinary course of events the vertebrate host is apparently never exposed to the free gametes, which are released in malarious blood only in the gut of the mosquito or on exposure to the atmosphere. It seemed reasonable to assume, therefore, that in natural conditions transmission proceeds in the absence of significant immunity against these stages in the life cycle of the parasite. By vaccinating with and artificially inducing immunity against the free gamete we hoped to block fertilisation and hence prevent transmission of the malaria parasite to the mosquito.

Blood from chickens with high (40–60%) but increasing parasitaemias of *P. gallinaceum* was drawn from the heart and washed immediately in 50 volumes of suspended animation (SA) medium (0.21% Tris; 0.96% NaCl; 0.2% glucose, adjusted to pH 7.4). Suspension of blood in SA medium facilitates removal of factors interfering with fertilisation and transmission, which may be present in the plasma, while the release of malarial gametes (usually spontaneous on exposure of blood to air) is reversibly suppressed. The

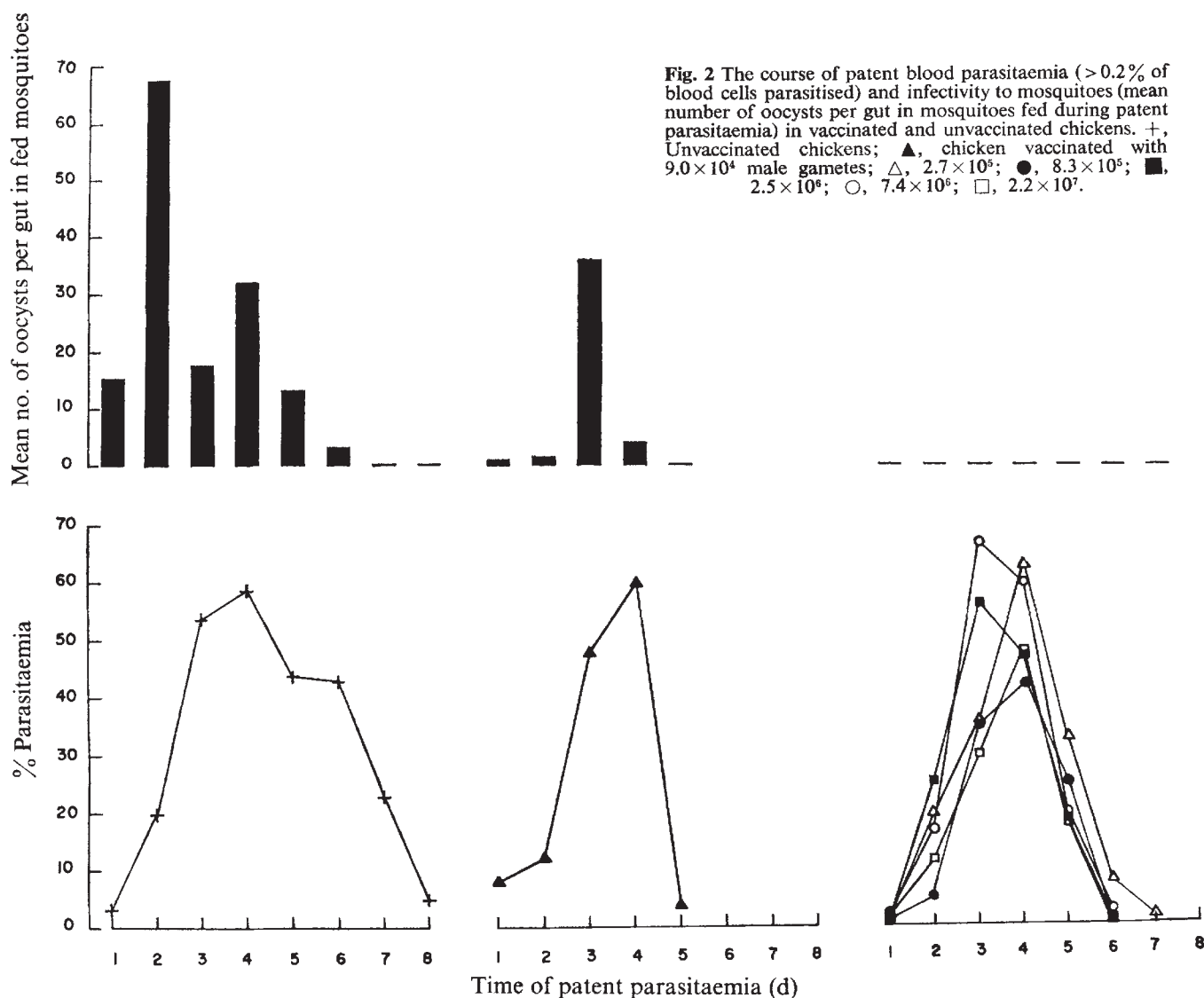
suspension of blood in SA medium was spun at 500g for 5 min and the packed cells were resuspended in a medium that induced the release of the gametes from their precursor cells in the blood (100 volumes of inactivated foetal bovine serum; 20 volumes of 1.46% NaHCO_3 ; 2.5 volumes of 5% NaCl, and 2.5 volumes of 10% glucose, adjusted to pH 8.0). The cells were incubated for 30 min at room temperature in 2 volumes of the gamete-releasing medium for each volume of packed cells to facilitate complete release of both male and female gametes, the suspension being readjusted to pH 8.0. After centrifugation of the cell suspension for 5 min at 500g the supernatant contained numerous gametes (male and female), some extracellular asexual parasites, red cells, white cells and acellular debris. The supernatant was then spun at 18,000g for 10 min. By visual estimation (comparison of the pellet with measured volumes of methylene blue in identical centrifuge tubes) the amount of material in the 18,000g pellet occupied between one and two thousandths of the packed cell volume of the blood from which it was derived. This was the material used for vaccination (Fig. 1). In view of the heterogeneous nature of the cell and subcellular material in this preparation it was difficult to quantify accurately the relative amounts of gamete and non-gamete material. The yield of male gametes was about 10% of that in the original blood. Male gametes represented about 50% and female gametes about 20% of the cells present. A detailed account of the method of collection of gametes will be published elsewhere.

The 18,000g pellet was resuspended in SA medium and X-irradiated with 20,000 rad. The concentration of male gametes in the suspension was counted using a haemocytometer and the material was diluted in SA medium by three-fold steps to give a series of six dilutions (Table 1). Six, 3-week-old, New Hampshire Red chickens were inoculated intravenously with three injections of the irradiated gametes (freshly prepared) in 1.5 ml of SA medium at weekly intervals. The total number of male gametes received by each chicken varied from 9.0×10^4 to 2.2×10^7 , representing the yield from 0.05 ml to 11.0 ml of blood at a 40–60% parasitaemia (Table 1). One week after the last inoculation of gametes the vaccinated chickens and one unvaccinated chicken of the same age were challenged with *P. gallinaceum*-infected blood. Peak parasitaemias of 40–60% were achieved in all vaccinated chickens compared with a peak parasitaemia of 58% in the control. A fresh batch of *Aedes aegypti* mosquitoes was fed daily on each chicken throughout its period of patent parasitaemia (defined here as 0.2% or more of blood cells parasitised). Seven days after feeding, 10 mosquitoes from each batch were dissected and their guts were examined for oocysts. The course of patent parasitaemia and infectivity to mosquitoes in experimental and control chickens are shown in Fig. 2. The total number of oocysts produced and the mean number of

Table 1 Oocyst production in mosquitoes fed on vaccinated and unvaccinated chickens

Total immunising dose of male gametes	Volume of parasitised blood from which the gametes were prepared (ml)	Mean no. of oocysts per mosquito gut during the period of patent blood infection	Total no. of oocysts produced throughout the course of infection by feeding 10 mosquitoes per d
Unvaccinated	—	25.2	2,267
9.0×10^4	0.05	8.2	411
2.7×10^5	0.16	0.00	0
8.3×10^5	0.47	0.01	1
2.5×10^6	1.4	0.00	0
7.4×10^6	3.8	0.01	1
2.2×10^7	11.0	0.00	0

Each chicken was vaccinated intravenously at three weekly intervals, the same amount of X-irradiated gamete containing material being inoculated each week. The total amount received by each chicken is indicated in terms of the number of male gametes in the three inocula and the volume of parasitised blood from which the preparations were derived. A single unvaccinated chicken was used as a control. After challenge with live parasites a fresh batch of mosquitoes was fed daily on each chicken during the course of patent blood parasitaemia (>0.2% of blood cells parasitised). Seven days after feeding ten mosquitoes from each batch were dissected and the oocysts on each mosquito midgut were counted.



oocysts per mosquito over the total period of patent infection in each chicken is shown in Table 1.

In a normal infection of *P. gallinaceum* in the chicken the total number of oocysts produced by feeding 10 *A. aegypti* per day throughout the course of infection lies in the range of 2,000–4,000. In our experiment the mosquitoes fed on the unvaccinated chicken yielded more than 2,000 oocysts, representing an average of about 25 oocysts per mosquito during the period of transmission by mosquitoes. By contrast, all but the chicken vaccinated with the fewest gametes were almost totally uninfected to mosquitoes throughout their period of patent infection, oocyst counts being reduced by 99.9–100%. Even for the chicken vaccinated with the fewest number of gametes (9×10^4 gametes equivalent to the yield of 0.05 ml of blood) infectivity, as measured by oocyst production, was reduced by 80% below that of the control. Similar results have been obtained using comparatively highly purified preparations of female gametes as a vaccine and with highly purified male gametes.

Oocyst counts in infected anopheline mosquitoes in areas of holoendemic human malaria are generally less than 10 per gut⁸. In principle the level of suppression of oocyst production achieved in our study should lead to considerable reductions in the infectivity of vectors of human malaria if the same response to gamete immunisation could be

reproduced successfully in exposed human populations.

We have demonstrated that a transmission-blocking vaccine based on malarial gametes is easily prepared, uncomplicated in administration (effective without adjuvants) and highly potent in the *P. gallinaceum*–chicken system. But as in the case of other forms of antimalarial vaccines tested with animal systems the translation of a gamete-based vaccine into a realistic measure for combating human malaria faces formidable problems. Not only must the effectiveness of a gamete vaccine be demonstrated for human malarias, but the means of producing large quantities of suitable material must be found. Nevertheless, if satisfactory progress is made in these areas we believe that a vaccine based on the free gametes of the malaria parasite may have considerable significance for the prospects of malaria control or eradication.

The concepts arising from this study may have relevance beyond their application to malaria. In most vector-borne protozoal or helminthic diseases (for example, malaria, babesiosis, trypanosomiasis, leishmaniasis and filariasis) the disease organisms undergo marked biological transformations after their entry into the vector. It is possible that in some instances vaccination of the host with parasite material from the earliest stages of transformation in the vector may reduce transmission of the disease.

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Induction of autoantibodies to red blood cells by polyclonal B-cell activators

THE ability to distinguish self from non-self is a fundamental property of the lymphoid system. One hypothesis for immunocyte triggering¹ proposes that the encounter between antigen and immunoglobulin receptors results in irreversible tolerance, whereas activation requires additional signals, thus ensuring that tolerance to self-antigens will always be the first and easiest event to achieve. An alternative view is that the self–non-self discrimination is carried out by T cells^{2,3}, whereas B cells could never be made tolerant to thymus-dependent antigens, including autologous antigens, because these antigens cannot deliver any signal to B cells by interacting with the Ig or any other B-cell receptor^{4,5}. Experimental support for this notion has been obtained by the demonstration that tolerance to autologous antigens can be broken by the injection of cross-reacting antigens⁶ and that there are antigen-binding human B cells able to bind human thyroglobulin³. To distinguish between the two hypotheses, it is necessary to determine whether tolerance to self-antigens exists at the B-cell level. This can be done by treating lymphocytes with polyclonal B-cell activators (PBA) and studying antibody formation against autoantigens, for PBA are competent to activate B cells of any specificity and thus to reveal the total V gene repertoire of the B cells. We have activated bovine spleen cells in culture with PBA (lipopolysaccharide (LPS) from *Escherichia coli* 055 : B5 and purified protein derivative (PPD) of tuberculin) and studied the number of plaque-forming cells (PFC) appearing against autologous red cells and heterologous red cells coated with the hapten NNP. In all experiments, the spleen cells and the red cells were derived from the same animal. We found that PBA-activated bovine lymphocytes synthesised autoantibodies against their own red cells.

As shown in Fig. 1, which represents the results from three cows, there was a response measured as thymidine incorporation to different concentrations of LPS, although to a lesser extent than with mouse spleen cells. But LPS induced activation, as determined by an increased thymidine incorporation, was highly significant, being three to six times the background values.

To study induction of autoantibody synthesis, bovine spleen cells were cultured in serum-free medium and stimulated with different concentrations of LPS and in some experiments also with PPD. After 2 d PFC against autologous red cells and hapten (NNP)-coated sheep red cells were counted. Bovine lymphocytes were used because bovine red cells can be used in the plaque assay⁷. As Fig. 2 and

Table 1 show, antibodies were always induced to NNP-coated sheep red cells although there were fewer PFC than expected from mouse experiments. There was, however, an analogous increase of the number of PFC against untreated autologous red cells. There were usually fewer PFC against autologous cells than against NNP-SRC, but the two showed the same increase over background and the same dose–response curve.

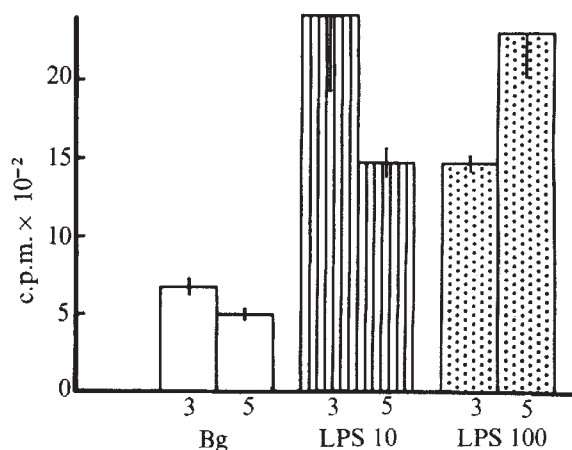
Thus, the polyclonal B-cell activators LPS and PPD could both induce the formation of autoantibodies by bovine spleen cells. These substances also induced thymidine uptake and plaque formation against NNP-coated sheep red cells.

It seemed possible that LPS and PPD would coat the red cells and that the PFC actually detected activity against these added foreign materials. But this possibility can be excluded on the basis of previous experiments⁸, because the concentrations of PBA used here were such that antibody formation against the PBA was completely inactivated, whereas polyclonal antibody synthesis was induced. Thus there must be resting B lymphocytes in bovine spleen cells that can be activated, by the addition of PBA, to synthesise antibodies able to lyse their own red cells.

These findings have far reaching implications for an understanding of the mechanism of self–non-self discrimination. They indicate that B cells are not normally tolerant to their own thymus-dependent self constituents. This agrees with findings on experimentally induced tolerance to a thymus-dependent protein antigen in mice, where completely tolerant animals had a normal complement of B lymphocytes able to produce antibodies against the tolerogen after activation by LPS (refs 9, 10).

The findings also have implications for an understanding of the mechanism of B-lymphocyte activation. According to the two-signal concept¹, signal one occurs after interaction between the antigen and the Ig receptor and results in an irreversible state of immunological tolerance. Only the simultaneous addition of both signals results in lymphocyte activation, whereas signal two alone has no effect. The alternative hypothesis suggests that only one non-specific signal activates the B lymphocytes⁹. This signal is not delivered by the Ig receptors, but rather by non-clonally distributed receptors for PBA, since B lympho-

Fig. 1 Induction of DNA synthesis with different concentrations of LPS in bovine lymphocytes in serum-free microcultures using 5×10^5 cells per well in 0.2 ml of Eagle's minimum essential medium in Earle's solution containing amino acids and antibiotics as described by Mishell and Dutton^{9,11}. The cultures were incubated at 37 °C as described before¹², and after 1–2 d each well received 1 μ Ci of 3 H-thymidine. The cultures were then collected 24 h later on glass fibre filters in a multiple harvester. The filters were dried, scintillation fluid was added and they were counted in a liquid scintillation counter. The figures below the bars denote the cow number in Table 1.



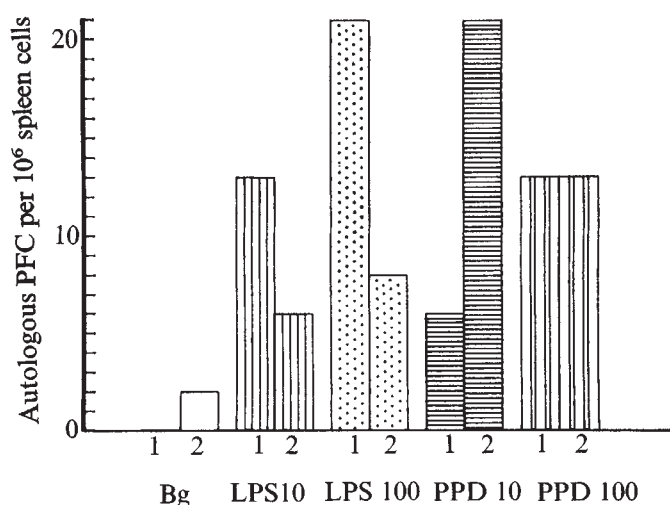


Fig. 2 Induction of autologous PFC in serum-free cultures with bovine lymphocytes stimulated with different concentrations of LPS and PPD. The numbers below the bars indicate cow number. Values are expressed as PFC per 10^6 lymphocytes. 1×10^7 – 2×10^7 spleen cells were cultured in 1 ml of medium in 3-cm plastic Petri dishes set up in triplicate. The cultures were incubated on a rocking platform for 2 d and thereafter the cells were assayed in a modified¹³ agar plaque assay¹⁴ using untreated autologous red cells or haptenated (NNP) sheep red cells. Haptenation was carried out as before¹³.

cytes from bovine spleens must necessarily have been in continuous contact with their own red cells, and in spite of this did not become irreversibly tolerant. The results suggest that interaction between Ig receptors and self antigens does not give a tolerogenic signal.

It has been pointed out that the self-non-self discrimination might be executed entirely at the T-cell level²⁻⁵. This is usually sufficient to maintain a state of self-non-self discrimination, because the autologous antigens are thymus dependent and thereby require cooperation between T and B cells for induction of an immune response. Since the self-reacting T cells would be absent, induction would not usually occur. Induction could be achieved, however, if the cells are given the relevant triggering stimulus delivered by different polyclonal B-cell activators as shown in this experimental system. This would usually occur as a consequence of exposure to PBA material, as happens in infections with organisms having or releasing PBA material. This is probably the reason for autoantibody formation in chronic infectious diseases such as mycoplasma infections,

leprosy, malaria and so on, where it has been shown clearly that the infectious organisms have polyclonal B-cell activating properties.

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Genetic control of the response of chicken leukocytes to a T-cell mitogen

THE way in which mitogens stimulate lymphocytes to divide in culture is not known. Some mitogens (for example, bacterial lipopolysaccharides) stimulate immunoglobulin-bearing lymphocytes to divide and secrete antibodies; others, such as concanavalin A (con A) or phytohemagglutinin (PHA), stimulate thymus-derived cells (T cells) to proliferate^{1,2}. An analysis of the mechanism of action of these mitogens may be helpful in deciding how specific lymphocytes respond to

Fig. 1 Responses of peripheral blood leukocytes from CB (---), WA (—), and (CB × WA)F₁ (- - -) birds to various doses of con A. Mean and range of responses of four birds from each strain are given for each dose. The response of a single bird is measured as the mean of ³H-thymidine uptake in triplicate 96-h microcultures, each culture containing 1.5×10^6 leukocytes in 200 μ l RPMI 1640 medium supplemented with penicillin (100 IU ml⁻¹), streptomycin (100 μ g ml⁻¹), and glutamine (2 mM) plus the specified dose of con A (Miles, Kankakee, Illinois). Cultures were set up in flat-bottomed wells of Falcon 3040 plates and collected as described previously⁶ 16 h after addition of ³H-thymidine (2 μ Ci in 50 μ l culture medium; specific activity 2 Ci mmol⁻¹) to each culture.

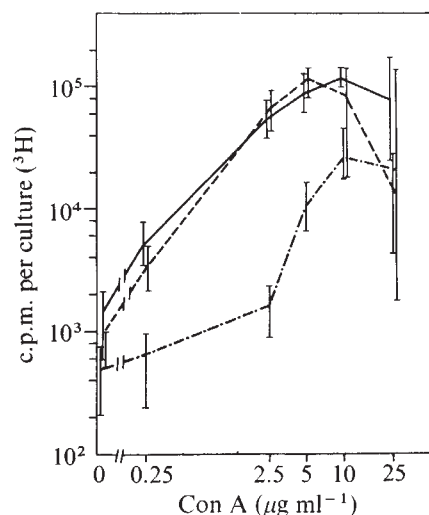


Table 1 Induction of PFC against NNP-coupled and autologous red blood cells by LPS after 4 d in serum-free cultures containing 2×10^7 cells each

Cow no.	Target cells	0	PFC per culture after addition of LPS		
			10 μ g	100 μ g	1,000 μ g
3	NNP-SRC	32	17	167	
	Autologous	0	0	7	
4	NNP-SRC	3	5	34	
	Autologous	0	1	6	
5	NNP-SRC	63	121	60	
	Autologous	0	5	3	
6	NNP-SRC	46	60	108	80
	Autologous	2	4	14	8
7	NNP-SRC	7	32	27	
	Autologous	6	24	31	

Bovine spleens were collected immediately after slaughter and the interior part was cut out. Blood samples were drawn simultaneously from the same animal.

antigen. We report here a case of genetic control of the ability of leukocytes to respond to a T-cell mitogen; lymphocytes from certain inbred chicken lines give a high proliferative response to con A, whereas those of other lines give a low response. The chicken provides a suitable model for studying such responses, in particular because it is easy to culture avian peripheral blood leukocytes and thus compare the results of mitogen stimulation directly with those obtained using human peripheral blood cells.

The CB and WA chicken lines used in these experiments are highly inbred^{3,4}. Peripheral blood leukocytes, prepared as described by Greaves *et al.*⁵, were cultured in the presence of varying doses of con A and PHA in microculture trays; after a fixed period (usually 3 d) ³H-thymidine was added to the cultures and after a further 16 h, the cells were collected and uptake of radioactivity (presumed to reflect DNA synthesis) was determined as described previously.⁶

Figure 1 shows the responses of leukocytes from adult (9–36-month-old) birds of the CB and WA strains, and of (CB×WA)F₁ hybrid birds, to various doses of con A. The responses of WA and F₁ birds are similar and are at least fivefold higher than the response of CB birds at all doses of con A except the (possibly toxic) highest tested. The responses were measured after 96 h, but, as Fig. 2 shows (for a selected dose of con A), the difference between the two strains can be seen throughout the response (that is, from 2–4 d).

Figure 2 also shows that leukocytes of the two strains respond equally well, or almost so, to a second T-cell mitogen, PHA; although only shown for a particular dose of PHA, this is true for a wide range of PHA concentrations. This fact, as well as providing a useful control for the survival of the leukocytes in culture, shows that the low response of the CB strain to con A is not caused by a defect in the ability of CB leukocytes to proliferate in culture (indeed, as shown previously, the response of CB leukocytes in a mixed leukocyte reaction is as good, or almost as good, as that of WA leukocytes⁶).

An analysis of the responses to con A of leukocytes from (CB×WA)F₂ and CB×(CB×WA) backcross birds is given in Fig. 3. Of 31 F₂ birds tested, 22 were high and 9 low responders. Of 8 backcross birds, 5 were high and 3 low responders. Most, including all the low responders, have been tested at least twice

Fig. 2 Time course of responses of peripheral blood leukocytes from CB and WA birds to con A (2.5 µg ml⁻¹) and PHA (1:100 diluted from PHA-M (Difco) stock). Cultures were set up in triplicate for each time point and pulsed for 16 hr with ³H-thymidine before being collected at the specified times, as described in the legend to Fig. 1. The source of CB (or WA) cells was a pool of leukocytes prepared from three CB (or WA) birds. Each point represents the mean of the triplicate values. Vertical bars represent the range of responses of three individual CB (I) and WA (J) birds to PHA (1:100 dilution), measured after 96 h (in a separate experiment, in identical conditions).

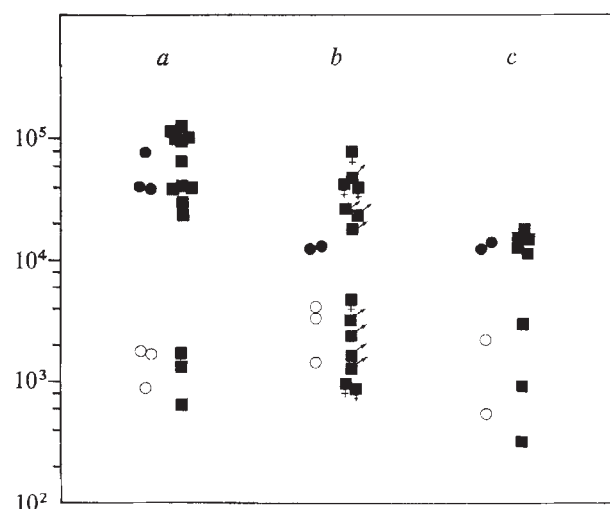
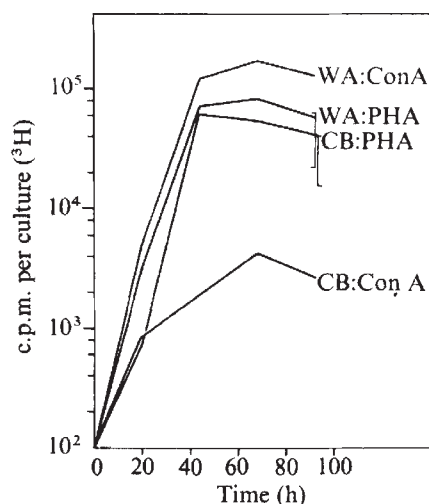


Fig. 3 Genetic control of the response of chicken peripheral blood leukocytes to con A. The responses of leukocytes from individual CB (○), WA (●) and (CB×WA)F₂ (■) or CB×(CB×WA)F₂ backcross (■) birds were measured after 96 h in culture, as described in the legend to Fig. 1, in three experiments. In the first (a), the responses of CB, WA and 14 randomly chosen (CB×WA)F₂ hens were measured; of the F₂ birds, 3 are low, and 11 high responders. In the second experiment (b), the responses of CB, WA and 14 selected F₂ birds (hens ♀ and cocks ♂) were measured; of the F₂ birds, 7 are high and 7 low responders. The birds were selected as high and low responders on the basis of previous experiments. In the third experiment (c), the responses of CB, WA, and 8 randomly chosen CB×(CB×WA) backcross birds were measured; of the backcross birds, 5 are high and 3 low responders. In each experiment, the responses to various doses of con A were measured; the values given are responses to the dose giving the optimum distinction between high and low responders (2.5, 2.5 and 0.25 µg ml⁻¹ of con A, respectively).

with consistent results. These findings indicate that a single, dominant gene controls the ability of peripheral blood leukocytes from the two strains to respond to con A in culture. We have provisionally called this locus *Mr1* (mitogen response 1) and its alleles *Mr1-hi* (WA) and *Mr1-lo* (CB).

Table 1 gives the results of tests for genetic linkage of the *Mr1* locus to the major histocompatibility (B) complex (which actually includes at least three closely linked loci⁷⁻⁹), and to the linked G1 and M1 immunoglobulin allotype loci^{10,11}.

Table 1 An autosomal locus, not part of the major histocompatibility or immunoglobulin gene complexes, controls the response of chicken peripheral blood leukocytes to con A

	High 21 (22.5)	Low 9 (7.5)	Total 30
B1/1	8 (9.0)	4 (3.0)	12
B1/9	9 (9.7)	4 (3.3)	13
B9/9	4 (3.7)	1 (1.3)	5
G1a/a	5 (4.5)	1 (1.5)	6
G1a/d	8 (10.5)	6 (3.5)	14
G1d/d	8 (7.5)	2 (2.5)	10
♂	10 (12)	6 (4)	16
♀	11 (10.5)	3 (3.5)	14

Phenotypes of 30 randomly chosen chickens derived from an F₂ cross between the inbred lines CB and WA. The B locus antigens (B1 or 9)^{3,4}, immunoglobulin G allotypes (G1a or d, which in this cross are associated with the immunoglobulin M allotypes M1a or M1c respectively)^{10,11} and sex of each bird were determined, as well as the reactivity (high or low) of its peripheral blood leukocytes towards con A. Numbers of birds of each phenotype are given, together with (in parentheses) expected numbers if there is no genetic linkage between loci controlling response to con A and the other listed factors. Phenotypes of the parental lines are: CB, B1/1, G1a/a; WA, B9/9, G1d/d.

The *Mrl* locus is not linked to these loci, nor is it sex linked or sex limited (Fig. 2). Tests for linkage of the *Mrl* locus to loci controlling a serum albumin polymorphism¹², feather colour¹³, and the C and E blood group antigens¹⁴ have also proved negative (J.R.L.P., V.M., and K. Hála, unpublished).

A single-gene effect on the ability of leukocytes to respond to con A could have a number of explanations, of which some may be tested directly. For example, the WA, but not the CB, strain may have a lymphocyte surface receptor which has a high affinity for con A, and which, on reaction with con A, triggers the cell to divide. An alternative, but equally likely, explanation is that a subpopulation of leukocytes (presumably of T lymphocytes²), whose size differs in the two strains, responds to con A. The *Mrl* marker may prove useful in identifying the putative receptor, or T-cell subpopulation, involved in the response.

Other cases of genetic difference in the ability to respond to mitogens have been described: lymphocytes from mice of the C3H/HeJ strain do not respond to lipopolysaccharides, whereas cells from other mouse strains respond well; the response is controlled by a single non-H-2-linked and non-Ig allotype-linked gene¹⁵. Small differences, shown to be genetic, in the ability of different mouse and rat strains to respond to the T-cell mitogens con A and PHA have been described^{16,17}; this differs from our results in that low responders to con A were also low responders to PHA, suggesting that polymorphism of a specific con A receptor is not an explanation for the observed differences. No genetic differences in the ability to respond to mitogens have been established in human populations, but that such differences may exist is clearly a point to consider when the results of mitogen stimulation assays of human T-cell function are being assessed.

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mixed lymphocyte macrophage culture (MLMC) interaction^{6,7}. The stimulating activity of the macrophages seems to be much greater than that of peripheral blood lymphocytes. Human Ia-like antigens (B-cell antigens) are strongly expressed on macrophages^{8,9}. In the mouse, the lymphocyte-activating determinants of the *M* locus as well as those of the *H-2* loci are also expressed on macrophages¹⁰. We now present evidence that the lymphocyte-activating determinants on human macrophages are also coded for by the *HLA-D* locus and that an antiserum against HLA-Dw2 (Ia-equivalent) or closely associated determinants can inhibit specifically the stimulatory properties of macrophages from Dw2-positive donors.

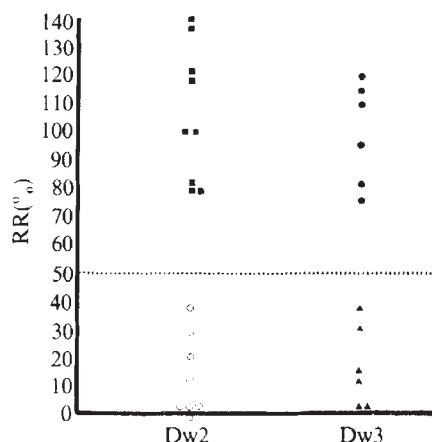
Leukocytes were separated from human peripheral blood by Ficoll-Isopaque flotation. Relatively pure macrophage populations were obtained by incubating the isolated leukocytes in plastic flasks for 8 h. The non-adherent cells, mainly lymphocytes, were decanted by repeated washing and the adherent cells were incubated for a further 7–8 d, after which the peripheral blood monocytes had matured into macrophages and the granulocytes had died out. The macrophages were collected by scraping the flask sides with a rubber policeman in calcium-free medium containing EDTA, and the cells were washed out in the same medium. The MLMC technique is described in the legend to Fig. 1. Stimulating macrophages were obtained from individuals known to be homozygous for a particular HLA-D determinant and used as typing cells against a panel of lymphocytes previously typed to be HLA-D heterozygous. In addition, macrophages were isolated from HLA-D heterozygotes and used as control stimulators for determination of the relative response. The anti-HLA-Dw2-associated antibody serum S ϕ W was produced by planned immunisation of HLA-A, HLA-B identical volunteers¹¹.

The pooled results from five experiments with macrophages from donors homozygous for either the Dw2 or Dw3 determinants are shown in Fig. 1. Macrophages homozygous for a particular HLA-D determinant stimulated rela-

Fig. 1 HLA-D typing results obtained with macrophages from HLA-D homozygous donors (Dw2 and Dw3). Responding lymphocytes were separated from donors previously typed to be Dw2 positive (○) or Dw2 negative (■); Dw3 positive (▲) or Dw3 negative (●). Responding cells (50×10^3) were mixed in combination with macrophages (10×10^3) in 0.2 ml of medium RPMI 1640 containing antibiotics and 20% human AB serum in the wells of microtitre plates. The plates were incubated for 5 d and collected after an 18-h pulse of ³H-thymidine. The results are expressed in relative response (RR) calculated from the formula:

$$RR = 100 \times \frac{\text{c.p.m. MLMC (homo. macrophages)} - \text{c.p.m. autologous culture}}{\text{c.p.m. MLMC (heteroz. macrophages)} - \text{c.p.m. autologous culture}}$$

The heterozygous macrophage control donors were always Dw2 or Dw3 negative respectively. Pooled data from five experiments.



Presence of HLA-D determinants on human macrophages

WHEN lymphocytes from two genetically dissimilar individuals are cultured *in vitro*, blast transformation and increased DNA synthesis occur in some of them; this is the mixed lymphocyte culture (MLC) interaction^{1,2}. In man, the MLC-activating determinants seem to be coded for by the genes of the HLA chromosome region, of which the HLA-D determinants seem to induce the strongest proliferation. HLA-D determinants are expressed on B cells³, skin cells⁴ and sperm⁵. Human macrophages can also activate allogeneic lymphocytes *in vitro* as detected in the

tively weakly ($RR < 40\%$) lymphocytes from heterozygous donors previously typed to possess the HLA-D determinant shared with the macrophage donor. This was the case for both the Dw2 and Dw3 determinants in all combinations tested (Fig. 1). Macrophages did, however, stimulate lymphocytes not sharing the particular HLA-D determinant to a degree comparable with that observed with stimulation by heterozygous macrophages ($RR > 70\%$), where a known two-haplotype incompatibility existed. A clear bimodality of the relative response distributions for the various responding cell donors, similar to that observed using homozygous lymphocytes as stimulating cells in typing MLC tests was observed for the macrophage stimulation for both specificities tested. In no experiment was it possible to demonstrate stimulation of lymphocytes by autologous macrophages, nor to demonstrate increased macrophage proliferation by culturing these cells in combination with mitomycin-treated allogeneic lymphocytes.

It seems therefore that the HLA-D determinants are found in the cell membrane of both lymphocytes and macrophages. This would account for the fact that macrophages from HLA-D homozygous donors failed to, or only feebly stimulated heterozygous lymphocytes sharing an HLA-D determinant. In other words, the HLA-D typing results obtained with homozygous macrophages or lymphocytes were completely congruent, and so a macrophage-specific differentiation antigen cannot be responsible for the positive response observed in MLMCs. In addition, little stimulation was observed in MLMCs between unrelated individuals typed to be HLA-D identical (unpublished observations).

We investigated whether antiserum shown specifically to inhibit the stimulating capacity of lymphocytes possessing the Dw2 determinant could also inhibit specifically the stimulating capacity of macrophages from Dw2-positive donors. This antiserum has been found to inhibit the stimulating capacity of lymphocytes possessing the Dw2 determinant, while inhibiting the responding cells to a much lesser degree¹². MLMCs were established in medium supplemented with either 20% normal human AB serum or a 1:1 mixture of the antiserum in normal AB serum. The antiserum had a significant inhibitory effect ($> 50\%$ reduction compared with controls) on all the stimulating macrophages possessing the Dw2 determinant (Fig. 2), but inhibited

Dw2-negative cells only slightly. The inhibiting effect of macrophages from individuals shown to be homozygous for the Dw2 specificity was particularly pronounced (Fig. 2). The stimulating properties of homozygous cells of other specificities however, were not inhibited significantly. The results of these inhibition experiments support the assumption that identical or closely linked stimulating structures (HLA-D) exist on macrophages as well as on lymphocytes.

The macrophage has a well known helper function in MLC, and cultures devoid of such cells do not give a positive response¹³. No specificity for this function has been demonstrated in human MLC, and the necessary macrophages can be either autologous or allogeneic to the responding cell. This helper function can be replaced by cell-free supernatants derived from adherent cells¹⁴, but not by mercaptoethanol⁷. Macrophages are also important in the presentation of mitogens and soluble antigens to T cells^{15,16}, and in the mouse effective cooperation requires identity between the macrophages and the T cells at the H-2 I region¹⁷, and therefore possibly the Ia determinants. It has been suggested that alteration of Ia determinants by antigen is the stimulatory signal in lymphocyte activation¹⁸. Our finding that the HLA-D determinants are present on human macrophages may indicate a similar function for HLA-D in man. The ability of macrophages to stimulate allogeneic lymphocytes, however, is probably a result of direct interaction between the stimulating determinants in the macrophage cell membrane and the responding lymphocytes, and not to a helper function *per se*, although this ability may be part of a more general T-cell activation mechanism.

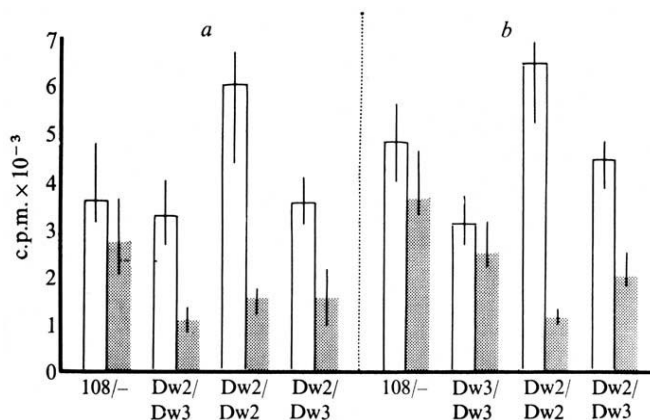
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Fig. 2 The inhibiting effect of an anti-HLA-Dw2-associated antiserum on macrophage stimulation. Open columns, c.p.m. of the cultures in 20% normal serum-supplemented medium; stippled columns, results in medium supplemented with 10% normal serum plus 10% antiserum. *a*, Responder (Dw3/oh) was the antiserum producer; *b*, an unrelated donor (Dw5/Dw6), not possessing the Dw2 determinant. The HLA-D typing data of the macrophage donors are shown. The results are the median and the range of triplicate cultures in c.p.m. Autologous responses have been subtracted.



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Origin of immunoglobulin-albumin complexes

THE propensity of human IgA and IgM monoclonal proteins to complex to endogenous serum proteins, such as albumin and α_1 antitrypsin (α_1 AT), has been previously established¹⁻³. When complexes of IgA-albumin and IgA- α_1 antitrypsin are isolated, albumin and α_1 AT are found on separate polymeric IgA molecules and not on monomeric IgA³. Another polypeptide chain, J chain, has also been shown to exist in polymeric IgA and IgM (refs 4 and 5) but not monomeric immunoglobulin. Furthermore, J chain is synthesised within IgA and IgM mouse plasma cells⁶. Therefore, it is important to determine

whether albumin is complexed to immunoglobulin as a step in intracellular biosynthesis or whether it is incorporated as a post-secretory event. We report here that IgG and IgA mouse plasmacytoma cells synthesise and secrete albumin complexed to immunoglobulin.

The synthesis of mouse immunoglobulin and albumin was studied using the incorporation of ^{14}C -leucine into plasmacytoma cells of the IgG (LPC-1), IgA (MOPC-315A, TEPC-15, HOPC-8) and IgM (MOPC-104E) class as described pre-

viously^{7,8}. All tumour lines were obtained from Litter Biometrics and maintained by serial subcutaneous passage in BALB/c mice. Their identity was confirmed by direct immunofluorescence and by light microscopy. After detergent lysis with 0.5% Nonidet P-40 at 4 °C for 15 min, protein from cell lysates and secretions was extracted using monospecific antiserum to mouse proteins above equivalence. The antisera to mouse IgG and IgA was subjected to affinity chromatography using a mouse albumin immunoabsorbent column. The anti-albumin antiserum was adsorbed using a mouse IgG immunoabsorbent column and shown not to precipitate radioiodinated mouse IgG double antibody precipitation. As a control for nonspecific precipitation, normal rabbit serum and an excess of goat anti-rabbit 7S globulin antiserum was added to an aliquot of all preparations. The immune precipitates were then analysed on sodium dodecyl sulphate (SDS) gels⁷.

The results of SDS-acrylamide (5%) electrophoresis of secretions from LPC-1 plasmacytoma cells, collected after 3 h of incubation with ^{14}C -leucine, are shown in Figs 1 and 2. Immunoprecipitation of IgG from these secretions followed by reduction yields two radioactive peaks, corresponding to γ and L chains (Fig. 1a). Complete depletion of IgG from secretions was shown by splitting the supernatant of the IgG precipitate into two fractions and testing aliquots, and reprecipitating with

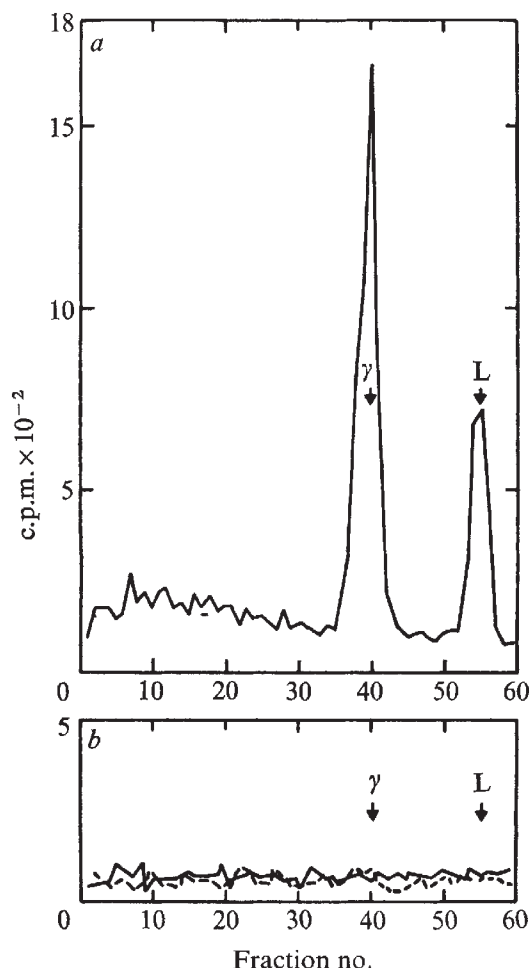
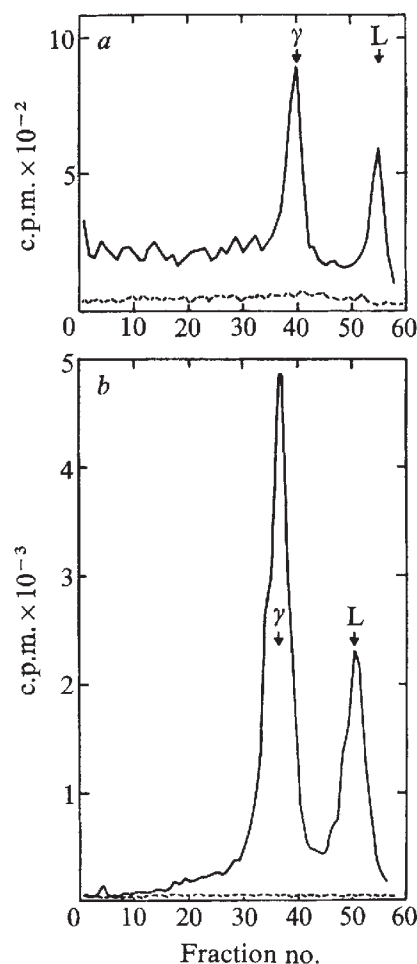


Fig. 1 SDS-polyacrylamide (5%) gel electrophoresis of reduced and alkylated radioactive, secreted mouse IgG (LPC-1). The LPC-1 cell suspension (15×10^6) was incubated in 3.0 ml of Eagle's medium minus leucine containing 10 μCi of ^{14}C -leucine at 325 mCi mmol^{-1} for 3 h at 37 °C. The cell suspension was chilled to 4 °C and made 0.06 M in iodoacetamide. After removal of the cells by centrifugation the supernatant was adjusted to contain 0.5% (w/v) Nonidet P-40 and dialysed against 0.5% Nonidet P-40 in PBS. The resultant secretion was precipitated in 0.06 M iodoacetamide by the addition above equivalence of a monospecific rabbit antiserum to mouse IgG adsorbed with mouse albumin. The precipitate was removed by centrifugation and the supernatant aliquoted into two fractions and further reacted with either rabbit anti-mouse IgG (200 μl) or rabbit anti-mouse albumin antiserum (200 μl) followed by the appropriate amount of mouse IgG (25 μg) or albumin (25 μg) to ensure complete precipitation. The precipitates were washed and dissolved in 2% sodium dodecyl sulphate (0.1 M iodoacetamide), 0.1 M sodium phosphate, pH 7.0, by heating at 100 °C for 2 min. Reduction was carried out with 0.5 M 2 ME by heating for 2 min at 100 °C and alkylation accomplished with excess iodoacetamide. Samples were electrophoresed in 0.1% SDS. The gels were sliced into 1-mm sections, eluted and counted and plotted with the top of the gel on the left-hand side of the figure. *a*, Reduced anti-IgG immune precipitate; *b*, supernatant of *a* reacted with an excess of either anti-IgG (—) or anti-albumin (---) antiserum. Arrows indicate marker proteins run simultaneously on separate gels. γ , Gamma heavy chains; L, light chains.

Fig. 2 SDS-polyacrylamide (5%) electrophoresis of ^{14}C -leucine-labelled LPC-1 cell secretions (3 h) precipitated with rabbit anti-albumin antiserum. Incubation with radioactive leucine and preparation of immune precipitate for SDS electrophoresis was as described in Fig. 1. *a*, Reduced and alkylated anti-albumin precipitate before (—) and after (---) adsorption of antiserum with mouse albumin; *b*, supernatant of the anti-albumin precipitate in *a* (—) further reacted with either anti-IgG (—) or anti-albumin (---) antiserum and analysed after reduction and alkylation. Marker proteins are indicated by arrows.



either an anti-IgG or anti-albumin antiserum (Fig. 1b). Similar results were obtained using double antibody precipitation with goat anti-rabbit gamma globulin. When anti-albumin antiserum is used to precipitate LPC-1 secretions, two peaks of radioactivity are again seen after reduction, which correspond to γ and L chains (Fig. 2a). This radioactive pattern is eliminated when the anti-albumin antiserum is adsorbed with the use of a mouse albumin immunoadsorbent column before precipitation of the secretion (Fig. 2a). A control of mouse serum added to secretions and precipitated with rabbit anti-mouse transferrin antiserum showed no precipitated radioactivity on SDS gels. Finally, the supernatant of the anti-albumin precipitate was totally depleted of radiolabelled albumin, while IgG γ and L chains were still present (Fig. 2b).

To demonstrate that anti-albumin antiserum specifically precipitated IgG associated with albumin, the amount of IgG (c.p.m.) in secretions before and after this immunoprecipitation was determined. IgG radioactivity in the secretion was depleted by 30–50%, using an anti-IgG antiserum for quantification. These experiments suggest that the precipitation of IgG by anti-albumin antiserum was due to complex formation between albumin and IgG, although no radioactive peak corresponding to albumin was seen on 5% SDS gels.

Since albumin could be secreted unlabelled by the IgG

Fig. 3 LPC-1 cell secretions externally labelled with ^{125}I . Secretions were collected after 3 h of incubation as in Fig. 1 and then externally labelled^{9,10} with ^{125}I and processed as given in legend to Fig. 1. Anti-IgG (a) and anti-albumin (b) antisera were used to react with secretions and the immune precipitates were electrophoresed, unreduced, on 5% SDS–polyacrylamide gels. Marker proteins, indicated by arrows, were obtained by radioiodinating isolated mouse IgG and albumin and precipitating these proteins in the presence of unlabelled secretions with monospecific antiserum. Alb, albumin.

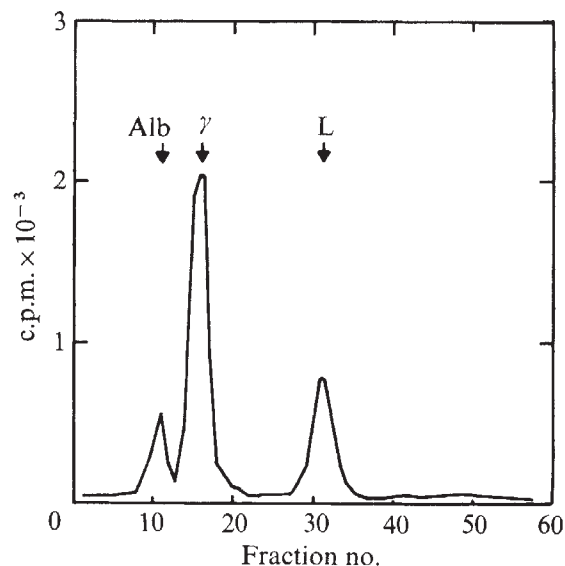
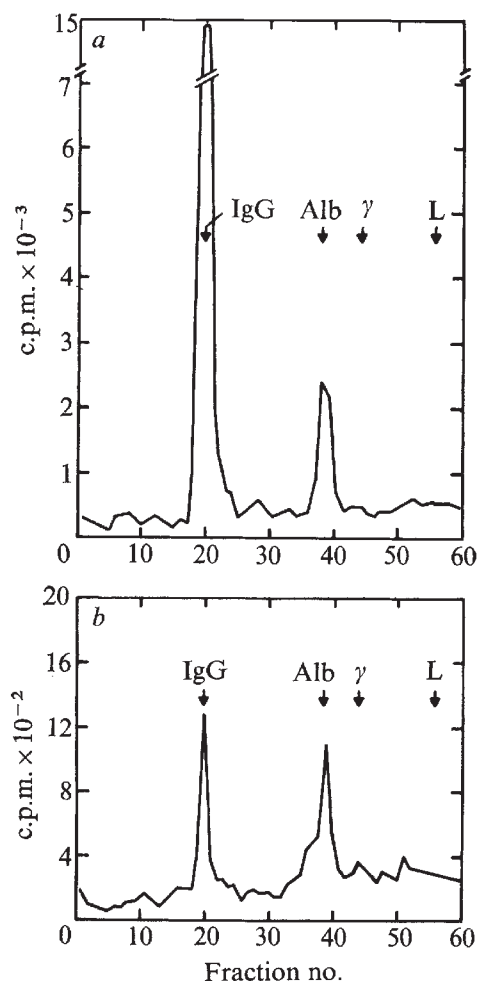


Fig. 4 Radiolabelled albumin secreted from LPC-1 cells after 5 h of incubation with ^{14}C -leucine. Conditions of incubation and handling of immune precipitates were as in Fig. 1. Secretions were precipitated with anti-albumin antiserum and reduced and alkylated and electrophoresed on 10% SDS–acrylamide gels with appropriate markers as in Fig. 1.

plasma cells, LPC-1 cell secretions were externally labelled^{9,10} with ^{125}I and after immunoprecipitation were analysed unreduced on 5% SDS gels. The anti-IgG precipitate of a 3-h secretion had two radioactive peaks corresponding to albumin and IgG (Fig. 3a). Similarly, the anti-albumin precipitate showed two peaks with analogous molecular weights on SDS gels (Fig. 3b), although the proportion of albumin to IgG differed from that obtained with antiserum to IgG. No discernible peaks were obtained on SDS gels of radioiodinated control secretions precipitated with anti-transferrin antiserum or normal rabbit serum and goat anti-rabbit gamma globulin. Planimetry of the graphs showed that the IgG–albumin complex was composed of 1 mol albumin per mol IgG and approximately 30% of the secreted IgG molecules were complexed to albumin. This accounts for the larger IgG peak seen with the IgG precipitate compared to the albumin precipitate. These experiments demonstrate that albumin is non-covalently bound to IgG and does not dissociate in the detergent, Nonidet P-40, although there is dissociation in SDS. Results obtained with three different IgA plasmacytomas were analogous to the IgG plasmacytoma, that is, albumin was secreted non-covalently bound to IgA. Neither albumin synthesis or secretion was found in IgM plasma cells.

To determine whether the albumin secreted from IgG and IgA plasma cells originated after biosynthesis or was adsorbed to the cell, lysates were examined after 3 h incubation with ^{14}C -leucine. In contrast to secretions, the cell lysates demonstrated a distinct radiolabelled peak for albumin, in addition to IgG, with anti-albumin antiserum. Since IgG and albumin were both radiolabelled in the complex present within the cell, it was possible that longer incubation periods would show ^{14}C -leucine incorporated into secreted albumin. The experiment in Fig. 4 depicts a 5-h LPC-1 secretion precipitated with antiserum to albumin and shows a third peak corresponding to albumin, in addition to γ and L chains. Furthermore, gel filtration of 5-h secretions on Biogel P200 equilibrated in 8 M urea demonstrated a component with a molecular weight of 68,000, antigenically identical to albumin. Similar experiments in three different IgA plasmacytomas demonstrate biosynthesis of albumin in these cells.

The complexing of albumin to IgG deserves further comment. Since no additional IgG or albumin is found in the supernatant after IgG is precipitated from secretions, this suggests that all of

the secreted albumin is complexed to IgG. One speculation is that the transport of albumin across certain membranes requires immunoglobulin. Furthermore, the albumin found in the complex is secreted by seniority—the older albumin molecules are secreted before the more newly synthesised (labelled) ones. If these complexes are present in the cell membrane, it is possible that the appearance of the complex in the culture media represents cell membrane turnover rather than direct secretion. The biological significance of the complexed albumin may be in the stabilisation of specific receptors on the cell membrane.

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Solitary cells and enzyme exchange in tetraparental mice

I REPORT here studies on tetraparental mice made with a greatly improved histochemical method for the enzyme β -glucuronidase. There were two unexpected findings: first, small clusters and solitary cells were found in many mosaic tissues of the tetraparental mice, and second, there was evidence that enzyme exchange occurs normally among cells.

Tetraparental mice are made by aggregating pairs of eight-cell embryos *in vitro*^{1,2}, and those I used were made from pairs of inbred strains whose tissues are known to differ in β -glucuronidase activity³. Thus the mosaic tissues of the tetraparental mice were composed of two intermingled populations of cells which could be distinguished microscopically in tissue sections stained histochemically for β -glucuronidase as originally demonstrated by Condamine *et al.*⁴.

Tetraparental mice (provided by Richard J. Mullen) were prepared from C57BL/6J or BALB/cWt (high glucuronidase) and C3H/HeJ or AKR/J (low glucuronidase) embryos⁵. Postnatal tetraparental mice 4 d, 9 d, 25 d, 10 months and 16 months old were used. Tissues from these mice and from homozygous controls were treated in parallel. Tissue specimens fixed in 4% formaldehyde were prepared for microscopy by a method involving several unusual steps, including incomplete dehydration, embedding in polyethylene glycol 400 distearate wax treated with NH_4HCO_3 and water, and staining of sections in a solution containing gelatin as well as the usual substrates (details in Fig. 1). This procedure permitted staining and demonstration of mosaicism in a much wider range of cell types than is possible with the method of Condamine *et al.*⁴. In many cells the staining was observed throughout the cytoplasm as well as in the lysosomes. This is in accord with findings that β -glucuronidase, which is a lysosomal enzyme, is also present outside the lysosomes^{6,7}.

In many tissues of tetraparental mice two populations of cells were readily distinguished by differences in staining intensity. The more intensely stained cells, which I shall call G cells, are assumed to be homozygous for *Gus*⁸, the wild-type allele for β -glucuronidase, and thus are assumed to arise from the C57BL/6 or BALB/c embryo. The more

lightly stained cells, termed g cells, are assumed to be homozygous for *Gus*^a, the allele resulting in lower glucuronidase activity³ and lower thermal stability⁹ of the enzyme; these cells are assumed to arise from the C3H or AKR embryo. Those tissues in which cells were not readily separable into two groups on the basis of staining intensity will be discussed later in this report.

The arrangement of G cells and g cells in a wide range of tissues revealed a thorough intermixture of both types, and a fairly widespread occurrence of small clusters of cells in many tissues (Figs 1–3). (I use 'cluster' to refer to a group of cells of one staining type surrounded by cells of the contrasting type.) There was striking variation in the ratio of the number of G cells to g cells as well as in size of clusters from animal to animal, from organ to organ within the same animal, and even from place to place within the same organ. Though large clusters were common, cells in small clusters (those containing fewer than 10 cells as viewed in a single section) made up between roughly 1% and more

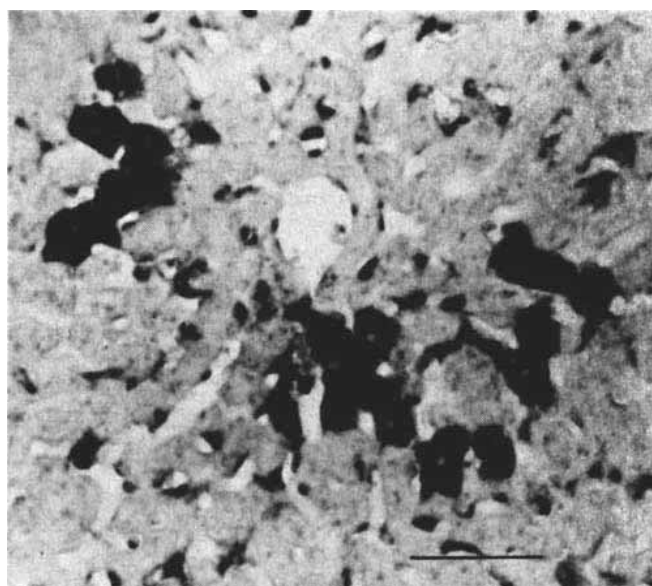


Fig. 1 Liver of 10-month-old tetraparental mouse showing mosaicism after staining for β -glucuronidase. About a dozen hepatocytes are intensely stained (G cells); the remaining hepatocytes—about 90% of all hepatocytes in this field—are lightly stained (g cells). (The much smaller, intensely stained cells are Kupffer cells.) Mice were fixed by intracardiac perfusion for 5 min with 4% formaldehyde (from paraformaldehyde) in a 0.15 M sodium phosphate buffer, pH 7.4. The whole mouse was then placed in a water-ice slurry. After 2–3 h, the organs were removed in a cold room at 4 °C and immersed in the same fixative at 0 °C for a total fixation time of 6 h. Specimens were rinsed at 0–4 °C for 2–5 d in several changes of 6% polyvinyl pyrrolidone (molecular weight 10,000) plus 10% sucrose (compare ref. 24), then dehydrated for 1–2 d at 0–4 °C in 98:2 acetone–water (several changes), and finally embedded in wax. Polyester wax (= polyethylene glycol 400 distearate)²⁵ at 40–50 °C was stirred for 3 h with 5% by weight of solid NH_4HCO_3 and filtered. Two millilitres of water at 80–90 °C were added with stirring to 100 ml of melted wax at 40–50 °C. Tissue specimens were embedded in the treated wax after infiltration for 0.5–2 d at 37–40 °C (several changes). Sections cut at 5–20 μm were mounted in a shallow pool of water on slides treated specially²⁶ with Mayer's albumen. After sections had dried for ~1 d, slides were dewaxed in acetone, then allowed to dry twice from 9:1 acetone–water (to promote section adhesion). Slides were stained at 37 °C in a solution of hexazotised pararosanilin, naphthol AS-BI glucuronide, and 5% gelatin (Eastman No. 1099); with gelatin, periods of staining up to 5 h were possible before a change of staining solution was necessary. Apart from the gelatin, the composition of the solution was the same as that described by Hayashi *et al.*²⁷. Because of the enormous range of glucuronidase concentrations, optimum staining time ranged from 20 min for some cell types (for example, tissue macrophages) to 25 h for others (for example, retinal neurones). The bar represents 50 μm .

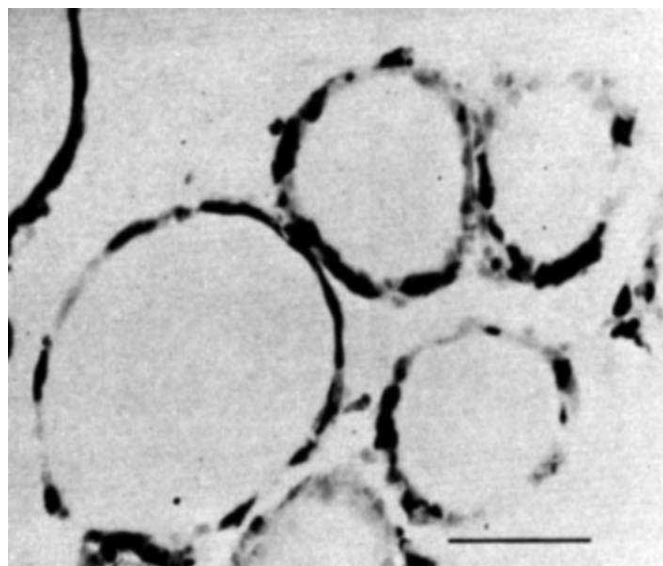


Fig. 2 Thyroid of 16-month-old tetraparental mouse. There are about a dozen G cells and about a dozen g cells in each of the four main follicles. The bar represents 50 μ m.

than 20% of the total cell population in many tissues. Small clusters were present in the following tissues of the 16-month-old tetraparental mouse and also in the same tissues of at least one of the three young mice (4, 9, and 25 d old): in the parenchyma of liver, pancreatic islets, exocrine pancreas, mucous and serous part of salivary gland, thyroid, parathyroid, adrenal cortex, seminal vesicle and Harderian gland; in the surface epithelium of salivary ducts, non-glandular stomach (basal layer of epithelium), glandular stomach, pylorus, small intestine, trachea, epididymis, choroid plexus, and ependyma; and in some nerve cell groups in the central nervous system.

The smallest 'clusters' consisted of single cells. The proportion of these cells ranged from a small fraction of a per cent up to a few per cent of the total cell population. Individual cells which seemed to be isolated in two dimensions when viewed in a single section were observed in most tissues listed above. The presence of truly solitary cells (those isolated in three dimensions) was verified by examination of serial sections of liver, salivary gland and duct, small intestine, thyroid, seminal vesicle, epididymis, Harderian gland and choroid plexus. Although small clusters and solitary cells have been noted previously⁸, this finding was unexpected: there have been several reports of much larger clusters in mosaic tissues—in the range of 10^4 to 10^8 or more cells per cluster⁹⁻¹⁴. This earlier work was based on methods of tissue analysis too crude for the detection of the small clusters which undoubtedly were present.

The presence of a solitary cell in a mosaic tissue means either that a pair of sister cells have separated after the cell division which produced them or that one member of the pair has died. The solitary cells found in this study may have migrated away from their sister cells at any stage of foetal or postnatal life; my observations provide no evidence on when migration occurred. One can, however, infer that cell migration in the region of large clusters was very slow¹⁵—no more than a few cell diameters of total migration by the average cell throughout postnatal life.

In the tissues discussed above the G cells and g cells formed two distinct populations readily distinguished by their staining intensities. Unfortunately, as previously mentioned, in some tissues many of the cells were of intermediate staining intensity and could not be assigned to either the G cell or g cell group. These included most of the lymphatic system, most connective tissues and many nerve cell groups in the central nervous system.

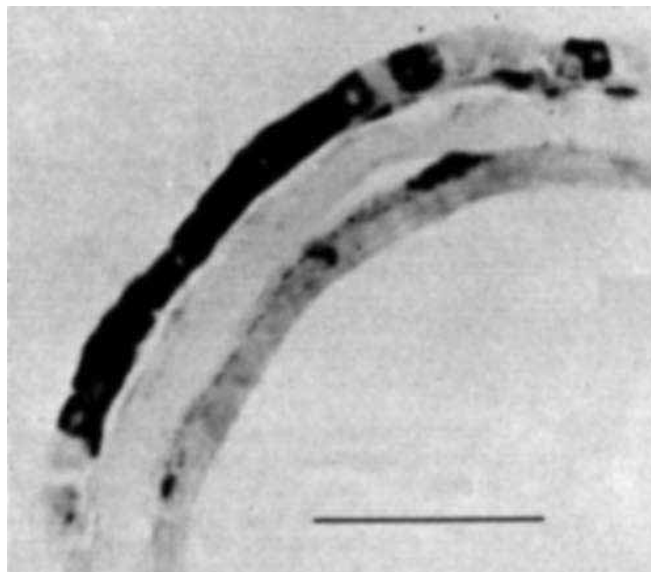
The differences in staining intensity between cells of tetraparental mice and cells of the same tissues in homozygous control mice showed some interesting regularities. The G cells of some tissues were stained less intensely in tetraparental mice than in the high-glucuronidase strains (C57BL/6 and BALB/c); this was observed in mucous and duct cells of salivary gland, stomach glandular epithelium, absorptive epithelium of small intestine, colon glandular epithelium, thyroid epithelium, adrenal cortical epithelium, renal tubular epithelium, Harderian gland epithelium, chondrocytes, smooth muscle cells, brown fat cells, some nerve cell bodies in the central nervous system, and Schwann cells (in trigeminal ganglion). Also the g cells of some tissues were stained more intensely in tetraparental mice than in the low-glucuronidase strains (C3H and AKR); this was observed in stomach glandular epithelium, absorptive epithelium of small intestine, colon glandular epithelium, adrenal cortical epithelium, renal tubular epithelium, epididymal epithelium, brown fat cells and choroid plexus.

These observations can be explained most simply by postulating a partial exchange of enzyme between G cells and g cells in tetraparental mice. An alternative explanation is that both the G cells and g cells of tetraparental mice have been influenced by the presence of cells of the opposite type to alter their level of endogenous enzyme. The hypothesis of enzyme exchange is supported by the preliminary finding that some g cells of tetraparental mice contain both *Gus^b* and *Gus^h* enzyme¹⁶. Evidence against the exchange of several non-lysosomal enzymes comes from the failure of attempts to detect hybrid molecules of these enzymes in a wide variety of tissues in tetraparental mice. Since hybrids could not be detected (except in skeletal muscle), the monomers of these enzymes apparently do not move from cell to cell (reviewed in ref. 17).

Change of phenotype by transfer of a substance from cell to cell in a mosaic tissue is a well known phenomenon. In the first recognised instance (described by Sturtevant in 1920; reviewed in ref. 18), the substance transferred between cells and found to be responsible for the observed effect proved to be kynurenine, a metabolite of low molecular weight. The findings reported here may be the first demonstration of exchange of an enzyme between cells in an intact animal.

Intercellular exchange of enzymes, particularly of

Fig. 3 Seminal vesicle of 16-month-old tetraparental mouse. There are two layers of secretory epithelium (simple, low columnar) separated by smooth muscle (almost unstained). One epithelial layer contains only g cells; the other layer, G cells and g cells. The bar represents 50 μ m.



lysosomal enzymes, may occur widely in tissues of ordinary animals. A similar suggestion has been made previously on other grounds¹⁹. Since many enzymes (including β -glucuronidase) are readily detectable in plasma and other extracellular fluids, these enzymes are available to be taken up by cells. Furthermore, the uptake of exogenous lysosomal enzymes administered experimentally is known to occur both in the intact animal²⁰ and in cell culture²¹; β -glucuronidase is among the enzymes for which this process has been demonstrated^{22,23}. Moreover, the incorporated enzyme has been shown in some instances to assume its normal function inside the cells that take it up^{20,21}. These observations, together with the findings presented here, support the view that exchange of glucuronidase (and other enzymes) among cells actually occurs as a normal function in a wide range of cell types.

The idea of using β -glucuronidase as a cell marker in tetraparental mice for studies of embryonic development was suggested to me by Richard L. Sidman.

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Hairbulb tyrosinase activity in oculocutaneous albinism

HUMAN oculocutaneous albinism (OCA), characterised by hypopigmentation of skin, hair and eyes, represents a heterogeneous group of at least four distinct autosomal recessive disorders^{1,2}. Tyrosinase-negative OCA and tyrosinase-positive OCA are the two most common forms and can be separated by genetic and clinical features¹. Tyrosinase-negative albinos have no obvious pigment in their skin, hair or eyes, and their plucked hairbulbs form no visible pigment after prolonged incubation in L-tyrosine or L-3,4-dihydroxyphenylalanine (L-dopa). In contrast, tyrosinase-positive albinos have variable, albeit minimal, amounts of pigment in their skin, hair and eyes, and their plucked hairbulbs form large amounts of visible pigment after prolonged incubation in L-tyrosine or L-dopa. The two less common forms of OCA are Hermansky-Pudlak syndrome and yellow mutant OCA. The specific defect in each form of OCA is unknown, and analysis of the biochemical steps in the formation of melanin have been hindered by a

lack of methods suitable for human studies. In this report, we describe an assay for quantifying tyrosinase activity in single human hairbulbs and give the results of the application of this technique to human oculocutaneous albinos.

Melanin formation occurs in the melanocytes, specialised cells found in the skin, hair follicle and eye³⁻⁵. Tyrosinase catalyses the hydroxylation of tyrosine to dopa and the oxidation of dopa to dopaquinone⁶. Dopaquinone then undergoes non-enzymatic oxidation and polymerisation to form melanin. Our assay measures the tyrosine hydroxylase activity of tyrosinase, determined by the rate of formation of ³HOH with the oxidation of L-3,5-³H-tyrosine to L-5-³H dopa, as described by Pomerantsev⁷.

Anagen (growing) hairbulbs plucked from the scalp were used as the source of enzyme. Hairbulbs contain a local concentration of melanocytes that remain in the bulb when a hair is plucked⁸, and animal studies have shown hairbulb tyrosinase to be present only during the anagen phase of the hair cycle^{9,10}.

The activity of normal hairbulb tyrosinase was tested from

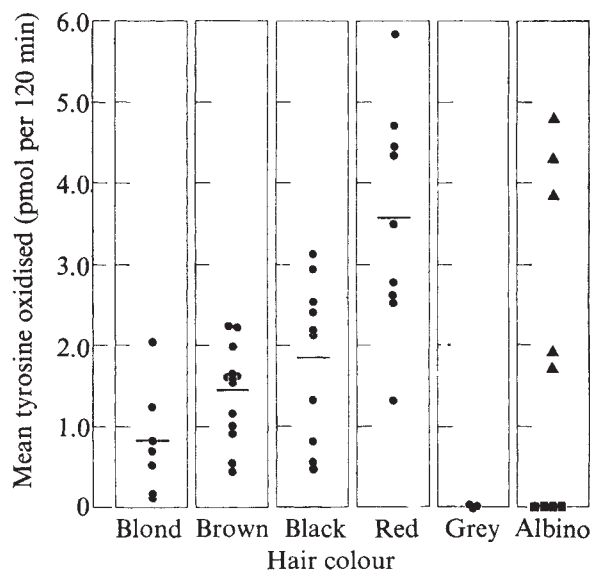


Fig. 1 Mean tyrosine oxidised per 120 min for hairbulbs from normal individuals with different hair colours and from oculocutaneous albinos. For albinos: ■, tyrosinase-negative and ▲, tyrosinase-positive. The overall mean is indicated by a line for blond, brown, black and red hair. Single anagen hairbulbs were identified visually, divided from the shaft at the shaft-sheath junction and incubated in tritiated tyrosine, dopa and Triton X-100. The incubation mixture used was determined to be optimum for single hairbulb tyrosinase quantification after many trials with different buffers, concentrations of substrate and cofactor and incubation volumes. The mixture consisted of L-3,5-³H-tyrosine, 0.2 nmol (1 Ci mmol⁻¹), L-dopa, 0.1 nmol; phosphate buffer, pH 6.8, 6 μ mol and Triton X-100, 0.5%, in a total volume of 0.06 ml, and was carried out at 37 °C in a shaking waterbath. Nonspecifically formed ³HOH in the stored L-3,5-³H-tyrosine was eliminated by evaporating the sample of tyrosine to dryness under a stream of nitrogen immediately before use. At 0, 60 and 120 min, a sample of the incubation mixture was placed on a Dowex-50W column equilibrated with 0.1 M citrate. The ³HOH was recovered from the column by washing with 0.8 ml of 0.1 M citrate and counted. The wash removed all tritiated water from the column, with no further activity above background recovered with a second wash. A blank consisting of the incubation mixture with no added hairbulbs was run with each assay. Values for the blank were subtracted from the hairbulb values before determining the amount of tyrosine oxidised. The reaction was linear throughout the 120-min incubation for normally pigmented and tyrosinase-positive albino hairbulbs. Boiled hairbulbs had no activity. Usually six to eight hairbulbs were assayed per subject, with enzyme activity expressed in pmol as the mean tyrosine oxidised per 120 min. The activity in the blanks was equivalent to 0.201 $\pm$ 0.152 (mean $\pm$ s.d.) for blond, 0.242 $\pm$ 0.089 for brown, 0.312 $\pm$ 0.153 for black, 0.255 $\pm$ 0.191 for red, 0.163 $\pm$ 0.172 for grey and 0.317 $\pm$ 0.235 for albino hairbulbs.

forty-two subjects with brown, black, blond, red and grey hair (Fig. 1). All subjects were under the age of 35 except for the three with grey-white hair, and no male was balding. The overall mean for seven subjects with blond hair was 0.821 pmol of tyrosine oxidised per 120 min with a range of 0.134–2.066 pmol per 120 min. Hair colour varied from light white-blond to bright golden blond, but there was no obvious association between degree of 'blondness' and enzyme activity. For thirteen subjects with different shades of medium brown hair a mean of 1.446 pmol of tyrosine was oxidised per 120 min with a range of 0.460–2.267 pmol per 120 min. On repeated testing, hairbulbs from two of the brown-haired subjects oxidised a mean of 1.620 ± 0.312 ($\pm$ s.d.) and 1.660 ± 0.266 pmol of tyrosine per 120 min. Ten subjects with black hair oxidised a mean of 1.881 pmol of tyrosine per 120 min with a range of 0.512–3.162 pmol per 120 min. Two of these subjects were black, and three were Oriental, but there was no association between enzyme activity and racial origin. Hairbulbs from nine subjects with red hair oxidised a mean of 3.596 pmol of tyrosine per 120 min, with a range of 1.352–5.862 pmol per 120 min. The variation was great between these subjects, but there was no obvious association between degree of 'redness' and enzyme activity. Grey-white hairbulbs from three subjects had little or no tyrosinase activity.

Four tyrosinase-negative albinos lacked enzyme activity in their hairbulbs (Fig. 1). Five tyrosinase-positive albinos could be divided according to activity into two groups (Fig. 1). Three had high enzyme activity, oxidising a mean of 4.819, 4.325 and 3.874 pmol of tyrosine per 120 min. These levels are equalled only by normal subjects with red hair. Beard anagen hairbulbs rather than scalp hairbulbs were used from one of the tyrosinase-positive albinos with high activity (4.325 pmol per 120 min) because of the inability to pluck adequate samples of scalp anagen hairbulbs. To verify the use of facial hair, beard hairbulbs from one of the black-haired and two of the brown-haired normal subjects were assayed and were found to have enzyme activity similar to or slightly reduced from the levels found in the same individuals' scalp hairbulbs. Two of the tyrosinase-positive albinos had moderate enzyme activity, oxidising a mean of 1.937 and 1.738 pmol of tyrosine per 120 min. These levels are similar to those for normal subjects with brown and black hair. Clinically, the two with moderate activity differed from those with high activity in that their hair was less pigmented and their visual acuity more reduced. Skin colour was similar in both groups. One of the albinos with moderate activity was 7 months pregnant, but had noticed no changes in skin, hair or eye pigmentation during the pregnancy. Pregnancy affects the hair growth cycle with more hair follicles in anagen¹¹, but the influence on tyrosinase activity is unknown.

The hydroxylation of tyrosine catalysed by tyrosinase utilises dopa as cofactor, and proceeds very slowly in its absence^{6,7}. To show that the hairbulb assay described here is dopa dependent, hairbulbs from a brown-haired subject and from a tyrosinase-positive albino were incubated in different

Table 2 Mean tyrosine oxidised per 120 min for hairbulbs from a brown haired subject

Experiment	Mean tyrosine oxidised per 120 min (pmol)
(A) Triton X-100 0.5% (regular assay)	1.620
Triton X-100 2.0%	1.520
Triton X-100 5.0%	0.986
No Triton	0.187
(B) Preincubation in 0.5% Triton	
Hairbulb removed at	
5 min	0.107
10 min	0.721
15 min	1.204
30 min	1.489

For experiment A, hairbulbs were incubated in L-3,5-³H-tyrosine, 0.2 nmol; L-dopa, 0.1 nmol; phosphate buffer, pH 6.8, 6 μ mol and Triton X-100, 0–5% in a total volume of 0.06 ml. For experiment B, hairbulbs were preincubated in 0.03 ml of 0.1 M phosphate buffer, pH 6.8, containing Triton X-100, 0.5%. After the hairbulbs were removed L-3,5-³H-tyrosine 0.2 nmol; and L-dopa, 0.1 nmol were added and the mixture assayed for tyrosinase activity.

concentrations of dopa, as shown in Table 1. When no dopa was added to the incubation mixture there was no oxidation of tyrosine during the 120-min incubation. Dopa concentrations above and below the usual assay concentration gave reduced levels of activity. The assay was clearly dopa dependent. The inhibition at a high dopa concentration was consistent with the action of dopa as a competitive inhibitor of tyrosinase, as shown with mouse and hamster melanoma tyrosinase^{6,7}.

All the above results were obtained using an incubation mixture containing 0.5% Triton X-100. To test the effect of different detergent concentrations, hairbulbs from a brown-haired subject were assayed in incubation mixtures containing 2%, 5% and no Triton X-100, as shown in Table 2 (experiment A). When no detergent was present, there was little enzyme activity. Triton X-100 (5%) inhibited the activity, and activity with 2% was similar to the regular 0.5%. Triton activation of tyrosinase has been demonstrated, but the exact mechanism of activation is unclear¹². In the system described here the Triton seemed to release the enzyme from the melanocyte, making it available for assay. This was shown by preincubating brown hairbulbs in 0.5% Triton for 5, 10, 15 and 30 min, after which the hairbulb was removed and the preincubation mixture without hairbulb was assayed for tyrosinase activity by the standard method (Table 2, experiment B). If Triton solubilised the tyrosinase, then the enzyme should have been present in the preincubation solution after the hairbulb had been removed. Hairbulbs preincubated with Triton for 5 min released little enzyme, as shown by an activity of only 0.107 pmol of tyrosine oxidised per 120 min for this preincubation mixture. Preincubation of hairbulbs for 10 and 15 min released progressively more enzyme and preincubation for 30 min released most of the available enzyme as shown by an activity of 1.489 pmol of tyrosine oxidised per 120 min for this preincubation mixture. Hairbulbs from this subject oxidised 1.620 pmol of tyrosine per 120 min in the standard assay.

Three conclusions can be drawn from this study. First, tyrosinase activity can be quantified in single anagen hairbulbs, and varies considerably among normal individuals with different hair colours and among normal individuals with the same hair colour. Although the trend was for more intensely pigmented hair (black and red) to have more hairbulb tyrosinase activity, there was no obvious association between intensity of a specific hair colour and tyrosinase activity. Further studies on the biochemical control of normal hair pigmentation are under way.

Second, tyrosinase-negative albino hairbulbs had no tyrosinase activity by this assay. This is the first quantitative demonstration of absent tyrosinase activity in this form of human OCA and supports the terminology of tyrosinase-negative used by Witkop¹. The absence of tyrosinase activity

Table 1 Mean tyrosine oxidised per 120 min for different dopa concentrations in incubation mixture

Dopa concentration (mM)	Mean tyrosine oxidised per 120 min (pmol)	
	Brown	Albino
0	0.000	0.000
1.67×10^{-4}	0.162	0.480
1.67×10^{-3}	1.620	4.325
1.67×10^{-2}	1.883	3.327
1.67×10^{-1}	0.945	—

The albino was a high activity tyrosine-positive albino. The incubation mixture contained 0, 0.01 nmol (1.67×10^{-4} mM), 0.1 nmol (1.67×10^{-3} mM), 1.0 nmol (1.67×10^{-2} mM) or 10 nmol (1.67×10^{-1} mM) of dopa in a total volume of 0.06 ml. The rest of the assay was as described in the text.

is consistent with the suggestion that this form of OCA is analogous to the *c* locus (*c/c*) albino animal¹, for the *c* locus is thought to control the synthesis of tyrosinase, with *c/c* animals being deficient in this enzyme¹³⁻¹⁵. Recently, Hearing¹² and Pomerantz and Li¹⁶ have shown that albino mouse eye and skin preparations retain small amounts of tyrosinase activity, 2-3% of that for pigmented eye and skin preparations. Our assay probably would not detect such residual activity in hairbulb preparations. There was definitely no stimulation of activity from tyrosinase-negative albino melanocytes, and there was no evidence for the presence of an inhibitor that could be dissociated from the enzyme by Triton. At present the evidence points to an absence of tyrosinase in tyrosinase-negative OCA.

Third, tyrosinase-positive hairbulbs from tyrosinase-positive albinos contain moderate to large amounts of tyrosinase that can be assayed readily with the system reported here. The defect in this form of OCA does not seem to be an absence of active enzyme, so other mechanisms must be considered. The structure of the tyrosinase-positive melanocyte or melanosome could be altered, but by electron microscopy these appear normal¹. Substrate availability or transport into the melanocyte could be deficient. The tyrosinase could be structurally abnormal with altered kinetics making it active in non-physiological conditions *in vitro* but inactive in physiological conditions *in vivo*. Finally, the activity of tyrosinase could be blocked by an inhibitor, for these have been described for melanoma tyrosinase¹⁷⁻²⁰. If an inhibitor is present, the release of tyrosinase from the melanocyte by Triton may also facilitate dissociation of the inhibitor from the enzyme.

There is insufficient information to show which of the above mechanisms is important in the production of tyrosinase-positive OCA. The fact that there seems to be genetic heterogeneity with two types of tyrosinase-positive albinos suggests that more than one mechanism may be active.

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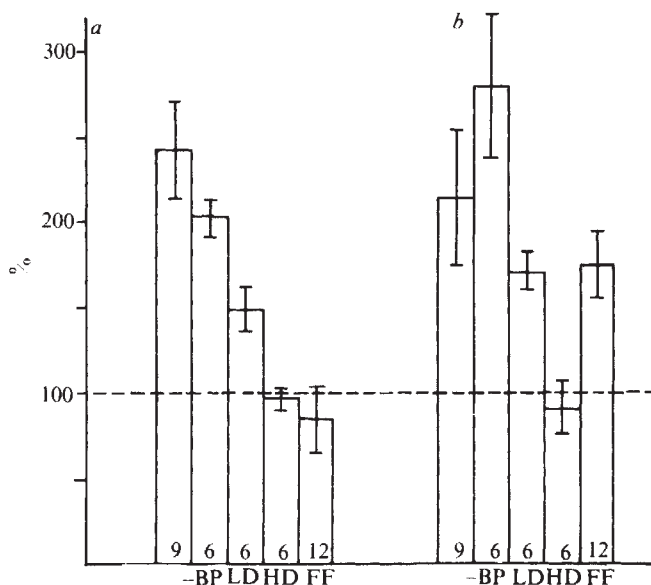
Evidence for inhibin-like activity in bovine follicular fluid

THE negative feedback action of testicular steroids on circulating levels of luteinising hormone (LH) is well known. It is less clear how the testis influences follicle-stimulating hormone (FSH). The existence of 'inhibin', a water-soluble testicular product that inhibits the secretion of FSH, was first postulated in 1932 (ref. 1); recently, inhibin activity has been detected in rete testis fluid², testicular tissue³, spermatozoa⁴ and seminal plasma⁵. Immunisation of rabbits with the inhibin fraction of bull seminal plasma has produced an antiserum which raised plasma FSH levels when injected into male and female rats⁶. It has been suggested⁷ that, in the male, inhibin may be produced by the Sertoli cells of the testis. If this is the case, one wonders if inhibin could also be produced by granulosa cells of the ovary. We have, therefore, looked for the presence of a specific FSH-suppressing agent in ovarian follicular fluid by comparing the effects of fluid and peripheral plasma on the levels of FSH and LH in newly castrated male rats.

A pool of follicular fluid was collected by aspiration from bovine follicles at various stages of the cycle. The concentrations of testosterone and 17 β -oestradiol in the follicular fluid, as measured by radioimmunoassay, were 23.5 and 73.1 ng ml⁻¹, respectively; these values agree with reported levels⁸. Bovine plasma, which has a similar protein composition to follicular fluid⁹, was obtained from a castrated bull and testosterone and 17 β -oestradiol were added to give concentrations of 25 and 75 ng ml⁻¹, respectively, in a low dose pool of plasma, and concentrations of 250 and 750 ng ml⁻¹, respectively, in a high dose pool.

Five groups of adult male rats (body weight 300-400 g), 6-12 animals per group, were castrated under ether anaesthesia. Approximately 1 ml of blood was collected from the cut spermatic artery at the time of castration (sample 1). Four of the groups of animals were injected intraperitoneally immediately after castration with either 0.25 ml bovine plasma, the low or

Fig. 1 Effect of injection of bovine plasma (BP), BP containing a low dose (LD, follicular fluid concentration) or high dose (HD, ten times follicular fluid concentration) of 17 β -oestradiol and testosterone, or bovine follicular fluid (FF) on plasma levels of FSH (a) and LH (b) in male rats 24 h after castration. All values are expressed as the percentage of precastration levels (means $\pm$ s.e.m., figures indicate numbers of animals).



high dose of steroids in plasma, or follicular fluid; and the animals in the fifth group served as untreated controls. Eight hours after castration a second injection of 0.5 ml of the appropriate fluid was administered subcutaneously. The animals were killed by decapitation 16 h after the second injection and blood was collected from the trunk (sample 2). LH and FSH were measured in samples 1 and 2 by radioimmunoassay¹⁰. The results of these estimations, expressed as the ratio of the concentration in sample 2 to that in sample 1, $\times 100\%$, are shown in Fig. 1.

The untreated controls and the group treated with bovine plasma did not differ significantly; both showed a two- to three-fold increase in plasma LH and FSH levels after castration. Treatment with the low dose of testosterone and 17β -oestradiol caused a significant decrease in plasma LH and FSH compared with bovine plasma-injected groups ($P < 0.05$ and $P < 0.02$, Student's *t* test); the high dose of steroids caused an even more marked suppression of both gonadotropins to precastration levels. Injection of follicular fluid caused a much greater suppression of plasma FSH than of plasma LH ($P < 0.01$); FSH being reduced to precastration levels, which were significantly lower ($P < 0.05$) than after treatment with the low dose of steroids. In contrast, plasma LH levels were comparable in the follicular fluid and low dose steroid groups. No significant ($P > 0.05$) differences in pituitary LH or FSH levels were detected between any of the five treatment groups.

To establish the non-steroidal, high molecular weight character of the FSH-suppressing agent in the follicular fluid, a second experiment was carried out. Groups of 6–12 animals were injected with steroid-free follicular fluid after castration as described above. LH and FSH were measured in plasma samples collected at the time of castration and 24 h later (Table 1). The effect on FSH levels of injected follicular fluid after ether extraction, charcoal treatment or ultrafiltration (molecular weight $> 10,000$) was not different from that of the untreated fluid. The suppression of the increase of LH in castrated rats after injection of untreated follicular fluid was abolished when steroids were removed from the fluid by these three treatments; remaining steroid concentrations were $< 1\%$ of the original concentrations in all preparations.

These observations suggest that bovine follicular fluid contains inhibin-like activity, which specifically suppresses FSH and cannot be ascribed to steroids, present in the fluid. The active compound has a molecular weight of over 10,000.

Table 1 Effect of injection of steroid-free bovine follicular fluid on plasma levels of LH and FSH in male rats 24 h after castration

Treatment*	n†	FSH	LH
None	12	215 $\pm$ 45	302 $\pm$ 72
Follicular fluid (FF)	12	99 $\pm$ 23‡	160 $\pm$ 89‡
FF, ether extracted	6	104 $\pm$ 42‡	219 $\pm$ 78
FF, charcoal treated	6	99 $\pm$ 24‡	294 $\pm$ 64
FF (molecular weight $> 10,000$)	6	90 $\pm$ 30‡	274 $\pm$ 189
FF (10,000 $>$ molecular weight $> 1,000$)	6	172 $\pm$ 69	247 $\pm$ 127

All results are given as the percentage of precastration levels in the same animals (means $\pm$ s.d.). Actual levels of FSH and LH at the time of castration were 366 ± 90 (s.d.) ng ml⁻¹ NIAMDD rat FSH RP-1 ($n = 48$) and 55.6 ± 22.9 (s.d.) ng ml⁻¹ NIAMDD rat LH RP-1 ($n = 48$), respectively.

*FF, Ether extracted: follicular fluid extracted six times with 1.5 volume of ether. FF, Charcoal treated: follicular fluid stirred with charcoal (1 mg ml⁻¹) for 30 min at room temperature and subsequently centrifuged at 12,000g for 30 min. FF (molecular weight $> 10,000$): follicular fluid diluted to 10 times its original volume with distilled water and subsequently reduced to its original volume by ultrafiltration (Amicon Diaflo PM 10, cutoff point at molecular weight of 10,000). FF (10,000 $>$ molecular weight $> 1,000$): the volume of the ultrafiltrate of first filtration was reduced to 0.9 times the original volume by ultrafiltration (Amicon Diaflo UM, cutoff point at molecular weight of 1,000).

†Number of animals.

‡Significantly different from untreated controls (Student's *t* test, $P < 0.05$).

Other indirect evidence, based on the inhibition of follicular development in immature mice injected with bovine follicular fluid¹¹, leads to a similar conclusion. Since it is unlikely that this substance could be produced by the one oocyte in the follicle¹², the granulosa cells are a more likely source.

It would be attractive to suppose that inhibin might act at the pituitary level, altering the ratio of FSH and LH secreted in response to releasing hormone. This might explain the changing FSH–LH ratio associated with puberty in both the male and female¹³ and would give a new dimension to our understanding of the gonadal feedback control of pituitary gonadotropin secretion.

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Prostaglandin mediation of collagenase-induced bone resorption

PATHOLOGICAL bone breakdown may be stimulated by some tissues proximal to bone and the release of collagenase by them may be one cause of this lysis. Inflamed gingivae, for example, have been shown to lyse collagen gels^{1,2} and are associated with the loss of bone in periodontal disease. The ability of human mammary carcinomas to break down bone *in vitro*^{3,4} has been shown to be caused, in part, by prostaglandin release by the tumours⁵ and, in part, by their release of an osteolytically active, non-dialysable material (unpublished results). The possibility that the latter might be collagenolytic in nature was suggested by reports that some neoplasms release collagenolytic activity *in vitro*^{6–9}. This prompted our present study of the mechanism of bone breakdown by exogenous collagenase. The investigation has shown that prostaglandin synthesis by bone cells is probably a necessary mediatory step in collagenase-induced bone resorption.

The addition of collagenase to tissue culture medium bathing ⁴⁵Ca-labelled neonatal mouse calvaria increases their release of ⁴⁵Ca (Table 1) and results in macroscopically obvious disruption of the calcified matrix, together with connective tissue lysis involving the fronto-parietal suture line and consequent physical separation of the two bones. An overall effect of this treatment is to cause the collapse of the bones on to the supporting grids. The addition of aspirin or indomethacin to the incubation medium containing collagenase causes inhibition of the release of ⁴⁵Ca, and although lysis of the connective tissue along the suture line

Table 1 Effect of collagenase on bone lysis determined in an assay using ^{45}Ca -labelled 4-d-old mouse calvaria as described previously⁹

Experiment	Concentration of collagenase (U ml^{-1})	Inhibitor	^{45}Ca release (test-control ratio)
<i>a</i>	5	—	2.34 ± 0.09
	10	—	2.58 ± 0.03
	15	—	3.31 ± 0.28
	15	Aspirin $10 \mu\text{g ml}^{-1}$	1.32 ± 0.04
	15	Indomethacin $1 \mu\text{g ml}^{-1}$	1.46 ± 0.17
<i>b</i>	5 (Live bone)	—	2.39 ± 0.44
	5 (Dead bone)	—	1.15 ± 0.10
<i>c</i>	—	SC19220 $25 \mu\text{g ml}^{-1}$	0.64 ± 0.04
	15	—	2.72 ± 0.39
	15	SC19220 $25 \mu\text{g ml}^{-1}$	0.73 ± 0.09

Bones were cultured in Biggers medium containing 5% heat-inactivated rabbit serum (Wellcome Reagents). Collagenase was from *Clostridium histolyticum* (Sigma, type III). Aspirin was added to the medium from a stock solution of pure acetylsalicylic acid in Biggers, and indomethacin was added from a stock solution in ethanol to give a final concentration of 0.01% ethanol. This concentration had no significant effect on collagenase activity in these assays. The bones in experiment *b* were killed by immersion in distilled water for 24 h. SC19220 was provided by Searle Laboratories, Illinois. The results are the mean of four determinations $\pm$ s.d.m.

of the frontal and parietal bones continues, no macroscopically obvious breakdown of the calcified matrix occurs. This treatment thus protects the bones from collapse other than at the suture line. No significant increase in ^{45}Ca release occurs if the bones are killed by immersion in distilled water before incubation with the enzyme, suggesting that an active cellular response is required, but severe weakening of the suture line is effected. Similar results are obtained using trypsin in place of collagenase.

Aspirin and indomethacin are inhibitors of prostaglandin synthesis¹⁰ and some prostaglandins are known to stimulate resorption in live bone *in vitro*¹¹. Although Brown and Pollock¹⁴ found that $1 \text{ mg indomethacin ml}^{-1}$ inhibited by 50% the action of collagenase on collagen as measured enzymatically, we found no such inhibition at drug concentrations below $100 \mu\text{g ml}^{-1}$ (Table 2). Similarly, in the bone assay system neither aspirin nor indomethacin prevented the separation of the bone along the suture lines. Our results suggest therefore that collagenase-induced osteolysis may be mediated by prostaglandins, an observation that is given support by the finding that SC19220, an antagonist of prostaglandin action¹⁵, is able to inhibit the collagenase-induced release of ^{45}Ca (Table 1). Further evidence that prostaglandins probably mediate, in part or whole, the action of collagenase on bone is provided by results which show that increases in the prostaglandin E content of incubation media occur in a dose-dependent manner on addition of collagenase (Table 3).

Raisz *et al.*¹⁶ have presented essentially similar evidence for prostaglandin mediation of complement-dependent stimulation of bone resorption. It seems likely that a similar mechanism of resorption is shared by collagenase, trypsin

and complement-sufficient heterologous serum, although stimulation of prostaglandin synthesis by the latter agent may depend on a different process. Inhibition of parathyroid hormone-stimulated bone resorption by aspirin¹⁷ is not of the same order as that found on bone resorption stimulated

Table 3 Effect of collagenase on prostaglandin release by bone *in vitro*

Experiment	Prostaglandin released per bone (ng PGE_2 Eq)
Control	< 0.3
Collagenase 15 U ml^{-1}	25.6 ± 20.0
Collagenase 5 U ml^{-1}	8.6 ± 4.5
Collagenase 15 U ml^{-1} + indomethacin $1 \mu\text{g ml}^{-1}$	< 0.3
Collagenase 15 U ml^{-1} + indomethacin 100 ng ml^{-1}	< 0.3

Unlabelled bones were used and the Biggers medium was supplemented with 0.5% bovine plasma albumin (twice crystallised, Armour Pharmaceuticals) instead of rabbit serum to avoid complications with prostaglandins present in the serum. Indomethacin was added in ethanol to give a final concentration of 0.01%; the same amount of ethanol was added to all other cultures. For radioimmunoassay, a neutral lipid extract was followed by ethyl acetate at pH 4.7 (citric acid). The residue on evaporation of this was assayed using an antiserum (gift from May and Baker) raised against prostaglandin E_2 (PGE_2). Cross reaction was found to be 66% for PGE_1 , but to be < 2% for PGs $\text{F}_{1\alpha}$, $\text{F}_{2\alpha}$, B_2 , A_1 and A_2 . The results are the mean of three determinations $\pm$ s.d.m.

by these agents. Indomethacin has been reported to have no significant inhibitory action on the stimulation by parathyroid hormone^{16,18}. We have also found (unpublished results) that aspirin and indomethacin do not significantly inhibit bone resorption stimulated by vitamin A or 1,25-dihydroxycholecalciferol (provided by Professor I. MacIntyre). It is probable that the resorption achieved by these agents is not as dependent on prostaglandin synthesis for its perpetuation. Since collagenase is found to be released by bone during parathyroid hormone-stimulated resorption¹⁹⁻²¹, however, prostaglandins may have some secondary function in physiologically normal osteolysis.

The digestion of bone matrix collagen by exogenous collagenase forms only one step in the highly coordinated process of bone resorption. To complete matrix breakdown, it is likely that other enzymes derived from the bone cells must act with collagenase extracellularly and that the resulting fragments must be endocytosed to undergo further breakdown. This is the same pattern of resorption as was suggested as taking place on parathyroid hormone or prostaglandin stimulation²², neither of which is markedly affected by indomethacin. It therefore seems probable that the essential difference between the mechanisms of resorption of collagenase and parathyroid hormone, for example,

Table 2 Effect of indomethacin and aspirin on collagenase activity

Concentration of collagenase (U ml^{-1})	Inhibitor	Enzyme activity
0.5	—	0.35
2.0	—	0.76
5.0	—	1.00
5.0	Aspirin $5 \mu\text{g ml}^{-1}$	1.03
5.0	Aspirin $50 \mu\text{g ml}^{-1}$	0.98
5.0	Indomethacin $1 \mu\text{g ml}^{-1}$	0.93
5.0	Indomethacin $100 \mu\text{g ml}^{-1}$	0.97

Enzyme activity is expressed as a ratio of the $5 \text{ U collagenase ml}^{-1}$ value. Acid-soluble collagen was prepared as described previously¹⁹. The method of acetylation and of collagenase assay followed that of Ginslow and McBride¹³. The reaction mixture for the assay contained $2 \text{ mg } ^{14}\text{C}$ -labelled collagen in a 1-ml volume, and the incubation time was 2 h at 37°C . Aspirin was added from a stock solution of pure acetylsalicylic acid in buffer. Indomethacin was added as a stock solution in ethanol and its effects were compared to 5.0 U ml^{-1} collagenase containing the same amount of ethanol.

is in the manner in which stimulation is achieved. It seems reasonable to assume that it is the initial digestion of collagen which causes the bone cell response to exogenous collagenase. The collagen fragments may cause a bone cell membrane reactions which leads to prostaglandin synthesis, and the resulting increased prostaglandin levels may cause normal bone resorption to ensue. Similar membrane effects have been suggested as being involved in complement-sufficient serum-induced bone resorption¹⁶.

Both prostaglandins and collagenase have been suggested as being responsible for the bone resorption associated with some tumours^{5-9,23,24}, inflamed gingival tissues²⁵ and dental cysts²⁶, and rheumatoid synovial tissue^{27,28}. The work implicating prostaglandin release from these tissues indicates that indomethacin may be a suitable agent for inhibition of the resorption. Our results suggest that, although the lytic effect of these tissues might be partially dependent on their release of collagenase, indomethacin may still be equally effective in preventing bone destruction.

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Simple model for treating evolution of multigene families

THE mechanism by which coincidental or horizontal evolution occurs in the multigene families (such as ribosomal RNA genes) represents one of the most challenging problems of molecular evolution^{1,2}. Smith³ and Black and Gibson⁴ put forward an elegant model of coincidental evolution, assuming homologous

but unequal crossing over. They have shown, using computer simulations, that repeated unequal crossing over results in the gradual loss of gene lineages, and that eventually the whole family becomes fixed by a single gene lineage. Here the population dynamics of gene lineages within a multigene family is analogous to the population dynamics of mutant genes within a finite Mendelian population. In this communication I show that a quantitative theory of unequal crossover fixation can be developed based on the mathematical theory of population genetics^{5,6}, treating the behaviour of mutant alleles in finite populations.

Following Smith³ and Black and Gibson⁴, I consider intra-chromosomal crossing over and concentrate on a single chromosomal lineage. Let us assume that there are n gene units within a family and that, initially, each unit is represented by a different gene lineage, so that there are n gene lineages at the start. To simplify the treatment, we assume that the mismatch between the two daughter chromosomes at the time of crossing over amounts to a shift of exactly one unit, so that one gene unit is either added or deleted through one unequal crossing over. We further assume that crossing over occurs at random along the n sites and the duplication and the deletion occur alternately, so that the total number of gene units is kept equal to the initial number, n . This corresponds to the action of natural selection holding the size of the gene family approximately constant. This model is illustrated in Fig. 1. Let us designate two consecutive crossovers (involving duplication and deletion) as one cycle of the process.

Now the problem is to analyse how gene lineages become fixed in the family or lost from it by continued unequal crossovers. This problem is quite similar to that of fixation or loss of mutant alleles in finite populations; the unequal crossover corresponds to sampling of genes at reproduction. Therefore, we can treat the frequency change of gene lineages in a multigene family as we treat the gene frequency change in standard population genetics, and in particular we can apply the diffusion model developed by Kimura⁷.

The frequency of a particular lineage in the family after a certain number of cycles of unequal crossovers is denoted by x . Note that, at the start, $x = 1/n$, because each lineage is singly represented in the family at the start. To apply the diffusion equation method in population genetics to our problem, we need to evaluate the mean ($M_{\Delta x}$) and the variance ($V_{\Delta x}$) of the frequency change per unit time (that is per cycle of unequal crossovers in this case). These quantities can be computed using Table 1. From the last two columns of Table 1, we can obtain $M_{\Delta x}$ and $V_{\Delta x}$.

$$M_{\Delta x} = \frac{1}{n}x(1-x) - \frac{1}{n}x(1-x) = 0 \quad (1)$$

$$V_{\Delta x} = \frac{1}{n^2}x(1-x) + \frac{1}{n^2}x(1-x) = \frac{2}{n^2}x(1-x)$$

Table 1 was constructed from the consideration that duplication or deletion by unequal crossover occurs at random on the chromosome, so that the probability that a particular lineage increases its size by one unit by duplication, or decreases its size by one unit by deletion, is in each case equal to frequency.

Once the mean and the variance of the frequency change per cycle are obtained, it is possible to apply Kimura's theory of gene frequency change. Note that the zero mean change ($M_{\Delta x} = 0$) in formula (1) implies that the population dynamics for selectively neutral mutants can be applied. In other words, my model is a diffusion process with a zero mean change and a non-zero variance as given in formula (1) and each gene lineage has equal chance of eventually becoming fixed in the family. Now, the dynamics of mutant frequency change in a finite population is a diffusion process with a mean $M_{\Delta x} = 0$ and a variance $V_{\Delta x} = x(1-x)/2N_e$ in case of neutral genes and in a population with effective size N_e . On the other hand, the

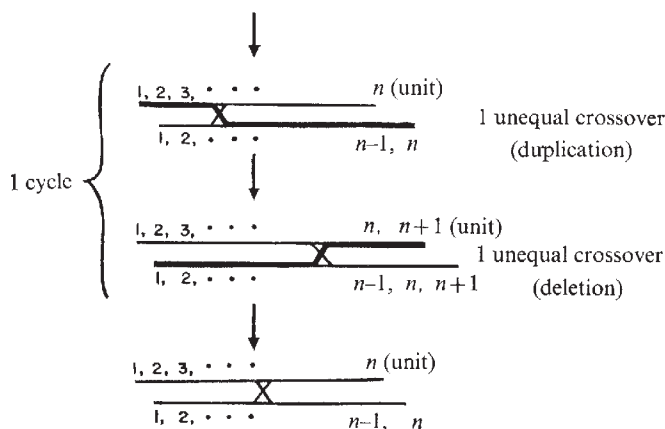


Fig. 1 Diagram illustrating the model.

crossover fixation time of one of the n lineages is equivalent to the time until fixation of a mutant allele excluding the cases of loss in population genetics. Kimura and Ohta⁸ have obtained the mean fixation time of a selectively neutral mutant in terms of the number of generations (excluding loss). Therefore by analogy, or by replacing N_e with $n^2/4$ in Kimura and Ohta formula, we get the mean of the crossover fixation time in terms of the number of cycles in the following form

$$t_1(p) = -\frac{1}{p} \{n^2(1-p) \log_e(1-p)\} \quad (2)$$

where p is the initial frequency of the gene lineage and equal to $1/n$. When n is large or p is small, this formula becomes approximately

$$t_1(p) \approx n^2 \quad (3)$$

Kimura and Ohta also obtained the variance of the time until fixation of the neutral mutant in finite populations⁹. Again, by replacing N_e with $n^2/4$ in their formula, we get

$$\text{var}(t_1) \approx 0.286n^4 \quad (4)$$

I have checked the above results by computer simulation and the agreements between the predicted and the observed values are generally satisfactory, both for the mean and the variance of crossover fixation time.

Pursuing the analogy with population genetics, it may be convenient to define a quantity similar to homozygosity. In fact, Smith³ used the quantity called the clonality in his simulation studies, which is equal to the sum of squares of frequencies of gene lineages, and which is equivalent to the homozygosity;

$$C = \sum_i x_i^2 \quad (5)$$

where the subscript i indicates the i th gene lineage. The expected change of clonality per one cycle of unequal crossovers can be calculated again analogously with that of homozygosity in finite populations.

$$\begin{aligned} E(\Delta C) &= E\left\{\sum_i (x_i + \Delta x_i)^2 - \sum_i x_i^2\right\} = E\left\{\sum_i (\Delta x_i)^2\right\} \\ &= (1-C) \frac{2}{n} \end{aligned} \quad (6)$$

where E stands for taking the expectation. This formula can be derived by using the formula for $V_{\Delta x}$ in (1) taking all lineages (that is, all i) of the family into account. Again, $n^2/2$ corresponds to $2N_e$ for the change of homozygosity in finite populations.

The present model may be an oversimplification of the actual process of crossover fixation; in particular it assumes

that the duplication and the deletion occurs by one unit. Smith³ has considered the cases where the duplication and deletion are allowed to vary by more than one unit and his simulation results suggest that this latitude accelerates the diffusion process. Although the exact theoretical treatment of such cases is difficult, very rough estimation can be done as follows. If the mean number of gene units duplicated or deleted by one unequal crossover is m , then one crossover should correspond to $m/2$ cycles of my model, provided that the arrangement of gene lineages is more or less random along the chromosome. By comparing Smith's results (Fig. 3 in ref. 3) with this estimation, it can be predicted that the random arrangement of gene lineages may be obtained when $m \approx n/10$ or when the allowed latitude (which is approximately equal to m) is roughly 10% of the total number of gene units. This

Table 1 Calculations for derivation of equation (1)

Duplication phase	Deletion phase	Total (1 cycle)
Change of x	Change of x	Change of x
Probability	Probability	Probability
$-\frac{1}{n}$	x	0
$+\frac{1}{n}$	x	x^2
0	$1-x$	$+\frac{1}{n}$
		$x(1-x)$
$-\frac{1}{n}$	x	$-\frac{1}{n}$
0	$1-x$	$x(1-x)$
		$(1-x)^2$

follows because the observed crossover fixation time in this case ($\approx 2,000$) corresponds to that predicted by the present model, that is $2/10 \times 10,000$. It should be noted here that Smith stopped his simulations at the clonality of 0.9 or when the frequency of a lineage reached about 0.95. The time required to reach this frequency is slightly smaller but not much less than the complete fixation time¹⁰.

I shall compare Smith's results with my prediction in more detail. He obtained the crossover fixation time of very roughly 8,000 for the case of latitude (m) = 1 and roughly 2,000 for $m = 5 \sim 25$. The predicted values by my model become for these cases: 20,000, 4,000, 2,000 and 800 crossovers corresponding to the four values of m (1, 5, 10 and 25). This would seem to imply that, for cases when $m < n/10$, the gene lineages are more clustered than random arrangement and hence the actual crossover fixation time is smaller than the predicted values. This is because the variance of the frequency change ($V_{\Delta x}$) gets larger by the correlated changes in such circumstances. If m is larger ($m > n/10$), the gene lineages become more dispersed than random arrangement and the theory gives an underestimation.

The analyses in this communication are not complete, but they at least provide a theoretical basis for further analysis in quantitative terms of the mechanism of coincidental evolution of multigene families.

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Do increases in enzyme activities during muscle differentiation reflect expression of new genes?

TRANSITIONS from embryonic isoenzymic forms to mature adult forms are known to occur during *in vitro* myogenesis for creatine phosphokinase¹ (CPK) and other muscle enzymes². More recently, similar transitions have been shown to occur for myosin^{3,4}, and actin^{5,6}. Both CPK activity^{7,8} and amino acid incorporation into myosin⁹⁻¹¹ increase during the early stages of differentiation *in vitro* when cells fuse to form multinucleate myotubes, and it is tempting to assume that these increases require the production of adult form mRNA after activation of a "battery" of genes for myogenesis². Yaffe and Dym¹¹ have proposed a model of myogenesis in which mRNAs for characteristic muscle proteins are synthesised before cell fusion and stored in a "masked" form until activated by the fusion process, thus giving rise to simultaneous increases in muscle-specific protein synthesis and cell fusion. We describe here experiments which show large increases in CPK activity at the

Fig. 1 CPK isoenzyme patterns of extracts from 74 h (●) and 96 h (○) muscle cell cultures. A sample (50 μ l) of extract was subjected to electrophoresis at 4 °C on 10% polyacrylamide gels with 5% cross linkage¹² for 3 h at 3 mA per tube. Slices (1 mm) of the frozen gels were placed in standard assay mixture and assayed for CPK activity as described previously⁸. CPK specific activity is expressed as international units (1 IU converts 1 μ mol of creatine phosphate to creatine per min of incubation at 37 °C) per mg soluble protein in the original extract.

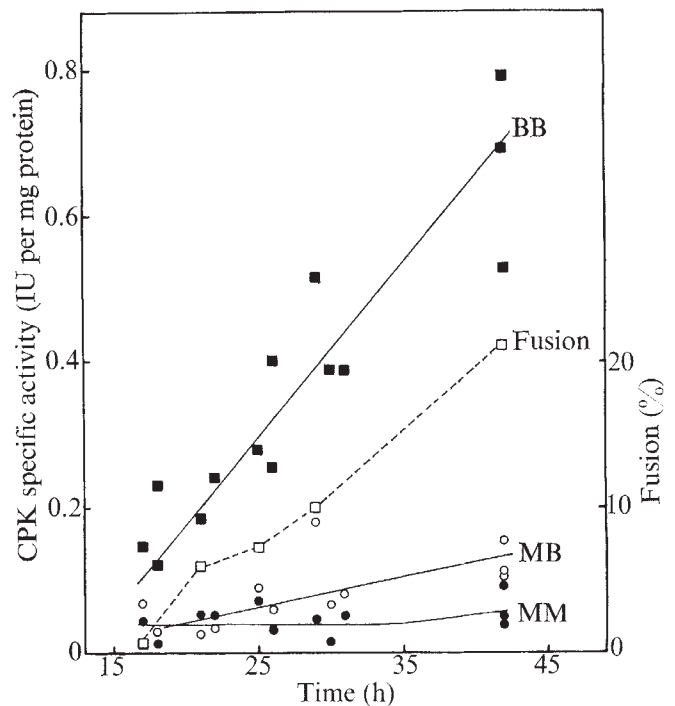
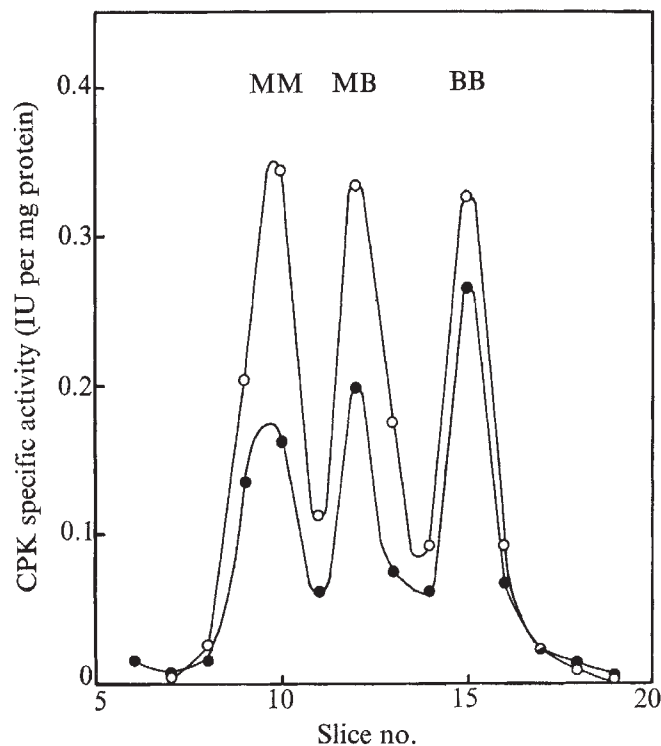


Fig. 2 Specific activities of the three isoenzymes of CPK (BB, ■; MB, ○; and MM, ●) during the first 2 d of culture. Cell fusion (□, broken line) was determined as previously described⁸. Each point represents a determination from a single gel and the combined data of four separate cell preparations are shown.

Creatine phosphokinase is a dimer of brain-type (B) or muscle-type (M) subunits and skeletal muscle differentiation is accompanied by a transition from BB isoenzyme, through the intermediate MB form, to the mature MM form. Figure 1 illustrates the resolution of the three forms on polyacrylamide gels and the continuing transition between 72 h and 96 h of culture. These results are consistent with those obtained by qualitative staining methods involving fluorescence¹ or colour² changes associated with enzyme-coupled reactions. Until now, however, no method has been sufficiently sensitive to study isoenzyme changes during earlier stages of culture when increases in cell fusion and enzyme specific activity are just beginning.

Between 17 h and 42 h of culture, fusion increases from

time of early cell fusion which cannot result from activation of "masked" muscle-specific mRNAs and which do not require adult form mRNAs at all. Using a new technique which allows measurement of low levels of CPK isoenzymes, we have shown that the early increase in CPK activity during the first 2 d of culture is of the embryonic, or brain, form, and only later does the adult muscle form make a major contribution to the total increase in enzyme activity.

Embryonic chick skeletal muscle cells were prepared and grown as described previously¹². Freeze-thawed cell pellets were extracted with 10% sucrose and samples from the centrifuged extract were assayed for CPK activity and protein as described previously⁸ to determine specific activity. Isoenzymes were separated by discontinuous polyacrylamide gel electrophoresis¹³ and the total specific activity was distributed among the three forms according to the ratios of the peaks of activity obtained after slicing the gel. Appropriate control experiments show that recovery of activity from the gel slices is independent of enzyme concentration and is the same for muscle and brain forms.

1 to 20% (Fig. 2) and total CPK specific activity increases sixfold. As Fig. 2 shows, this increase is due almost entirely to the BB embryonic form of the enzyme, the mature and intermediate forms showing little change. Whether the simultaneous increase in cell fusion and BB-CPK activity is anything other than coincidental remains to be determined. Although we, and many others, have shown that apparently normal increases in CPK activity can occur at later stages in the absence of cell fusion¹², this has not yet been established for the early increase described here. Earlier⁸, we described a transient increase in CPK activity occurring at the time of the initial increase in percentage fusion and before the much larger increases in enzyme activity which occurred later. It is possible that that transient increase is related to the increase in the BB form described here, although direct comparison is not possible since we now use cells prepared differently which fuse and develop much earlier. In this respect, it is interesting that muscle-forming cultures of mouse teratocarcinoma cells show an increase in CPK at the time of myotube formation, followed by decrease, but without any isoenzyme transition from the BB form¹⁴.

The assumption that muscle-specific forms are also involved underlies experiments in which CPK activity and myosin synthesis were "superinduced" by actinomycin D during and shortly before cell fusion. These experiments have been used to support the model involving prefusion synthesis of "masked" mRNAs for protein characteristic of the differentiated state¹¹. We have been able to obtain stimulations of 30–50% of total CPK specific activity (and of total activity per culture) by exposing either 22-h or 42-h cultures to actinomycin D (Sigma, 1 $\mu\text{g ml}^{-1}$) for 4 h. Although an increase of this size specific for the MM or MB forms would be readily detected by our electrophoretic method at these stages, we have found no significant change in the isoenzyme pattern, suggesting that the stimulation is either nonspecific or involves only the BB form. It is fair to say, however, that Yaffe and Dym¹³ used rat cells, rather than chick, in rather different culture conditions and obtained larger stimulations by actinomycin D, but, at the very least, our results argue forcibly for the need to demonstrate the isoenzyme transition before conclusions in terms of stored mRNAs for muscle-specific molecules can be made.

In general, we would advise caution in interpreting data on increases in enzyme activities or synthesis during differentiation in terms of new gene expression until they are shown to be due to the authentic mature form of the protein. In view of reports of isoenzyme-like transitions in myosin heavy chains^{3,4}, these considerations are also applicable to the interpretation of early increases in myosin synthesis during differentiation^{10,11}, and may influence conclusions drawn from studies of myosin mRNA during myogenesis, since the myosin mRNA synthesis observed in early cultures^{9,15} may be of the form that is not muscle specific.

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Inhibitory effect of α -(1 $\rightarrow$ 6)-heterogalactan on oocyte maturation of starfish induced by 1-methyladenine

THE surface of the starfish oocyte is thought to carry receptors for 1-methyladenine (1-MA)^{1,2}, which induces maturation division by some mechanism which is at present little understood. Using α -(1 $\rightarrow$ 6)-heterogalactan (HGaln) and α -(1 $\rightarrow$ 6)-galacto-oligosaccharides, which strongly inhibit the induction of maturation division by 1-MA, we have now obtained evidence that the receptor for HGaln is on the cell surface. We found that in the presence of HGaln, 1-MA did not activate the cell surface.

HGaln is extracted from the fruiting bodies of *Lentinus edodes* and has a main chain of (1 $\rightarrow$ 6)-linked α -D-galactopyranose residues, parts of which are substituted in the 2-position with either single L-fucopyranose or D-mannopyranose residues³. HGaln gives one peak only on ultracentrifugation ($S = 1.55$), zone electrophoresis and gel filtration.

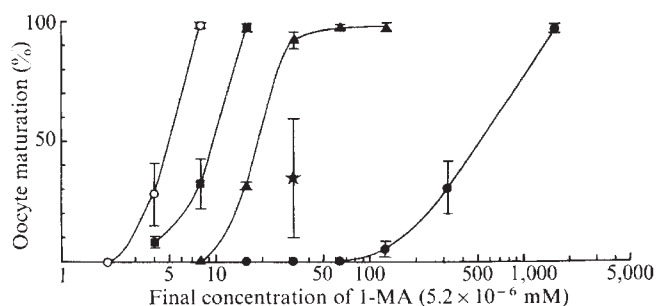


Fig. 1 Dose-response curves of the starfish oocytes to 1-MA in the presence of α -(1 $\rightarrow$ 6)-HGaln (FC, 0.83%) (●), or α -(1 $\rightarrow$ 6)-galactotetraose (FC, 0.83%) (▲), or in the absence of any sugars (○). ■, Dose-response of the washed oocytes by HASW after incubation with 0.83% α -(1 $\rightarrow$ 6)-HGaln for 30 min at 0–4 °C. Note the inhibitory effect of α -(1 $\rightarrow$ 6)-HGaln and α -(1 $\rightarrow$ 6)-galactotetraose on the oocyte maturation. At the star 192 $\times$ (5.2 $\times$ 10⁻⁶ mM) of 1-MA was mixed with 5% α -(1 $\rightarrow$ 6)-HGaln at the same volume for about 1 h at 0–4 °C, and 10 μ l of the mixture was added to 20 μ l of the starfish oocyte suspension in HASW.

Specimens of the starfish *Asterina pectinifera* were collected from Muro Beach, Japan, and kept in an aquarium at 0–4 °C. Ovaries were removed with forceps in calcium-free Herve's artificial seawater (HASW), and oocytes were prepared as before⁴. After incubation for 1 h in HASW at 0–4 °C, supernatant was decanted, and the oocytes were resuspended in HASW. This washing procedure was repeated to remove follicle cells. Although oocytes stored at 0–4 °C in HASW maintained their sensitivity to 1-MA at the same level for several days, it was necessary to test this sensitivity before each experiment in order to obtain quantitative results. Serial experiments were

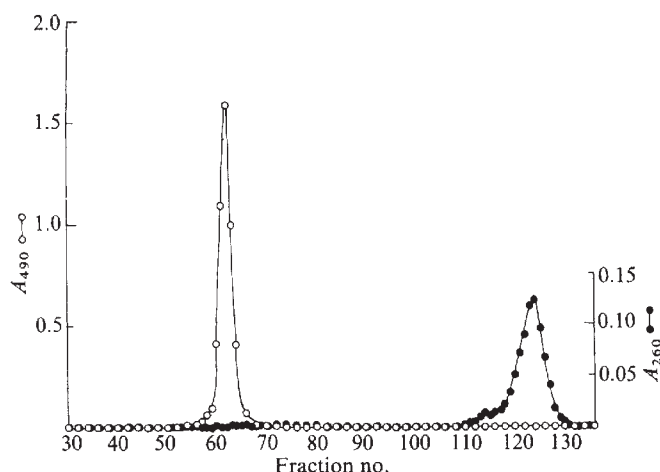


Fig. 2 Chromatography of the mixture of α -(1 $\rightarrow$ 6)-galactotetraose and 1-MA on Sephadex G-25. The column (2.64×85 cm) was washed with 500 ml of redistilled water and re-equilibrated with 1 l of 2/3 HASW. The applied sample contained 0.4 ml of 5% α -(1 $\rightarrow$ 6)-galactotetraose aqueous solution, 0.4 ml of 1 mM 1-MA aqueous solution and 0.8 ml of HASW. Elution was carried out with 2/3 HASW. Fractions (5 ml) were collected at a flow rate of 10 ml h^{-1} . $\circ$, A_{490} ; $\bullet$, carbohydrate measured by the phenol-sulphuric acid method at 490 nm.

performed with the oocytes of one animal. To detect the response of the starfish oocytes to 1-MA, 10 μl of 1-MA dissolved in redistilled water was added from a microcapillary pipette into 20 μl of oocyte suspension dispersed in HASW on

hole glasses (well size, 22×2 mm). The mixture was agitated thoroughly with a fine needle. Whole oocytes and those with broken germinal vesicles (OBGV) that had been induced spontaneously were counted under the microscope within 10 min. Breakdown of germinal vesicles began after 15 min of incubation and ended within 30 min of addition of 1-MA, independent of concentration. Therefore the hole glasses were covered with glass plates and after 30–40 min total OBGV was recorded. Each experiment was carried out three times and the percentage of oocyte maturation was calculated as OBGV induced when 1-MA was added to total oocytes. To test the inhibitory effect, 10 μl of the test solution dissolved in redistilled water was added to 40 μl of oocyte suspension in HASW and agitated. A sample of 10 μl of 1-MA solution in redistilled water was then added within 10 min.

As Fig. 1 shows, these oocytes underwent 100% maturation when 1-MA was added in a final concentration (FC) of more than $8 \times (5.2 \times 10^{-6} \text{ mM})$. On the other hand, maturation was completely inhibited by 0.83% (FC) heterogalactan in the presence of the same concentration of 1-MA (FC, $8 \times (5.2 \times 10^{-6} \text{ mM})$). FC of $1,500 \times (5.2 \times 10^{-6} \text{ mM})$ 1-MA was needed to induce 100% maturation in the presence of 0.83% α -(1 $\rightarrow$ 6)-HGaln.

The question is whether the inhibitory effect of HGaln was due to D-galactose, or D-mannose. It was also necessary to exclude nonspecific absorption of polysaccharides on the cell surface. We therefore tested for inhibition by other polysaccharides in experiments summarised in Table 1.

No inhibitory effect was detected with α -(1 $\rightarrow$ 6)-mannan isolated from the mutant *Saccharomyces cerevisiae* X2180 (ref. 5), Dextran T 40 (molecular weight 40,000, Pharmacia), or glycogen from rabbit liver (Sigma). This excludes nonspecific inhibition by polysaccharides of the induction of oocyte maturation by 1-MA. D-galactose, L-fucose and D-mannose

Table 1 Effect of the polysaccharides or monosaccharides that constitute α -(1 $\rightarrow$ 6)-HGaln, or oligosaccharides from the partial hydrolysate, on oocyte maturation induced by 1-MA in *Asterina pectinifera*

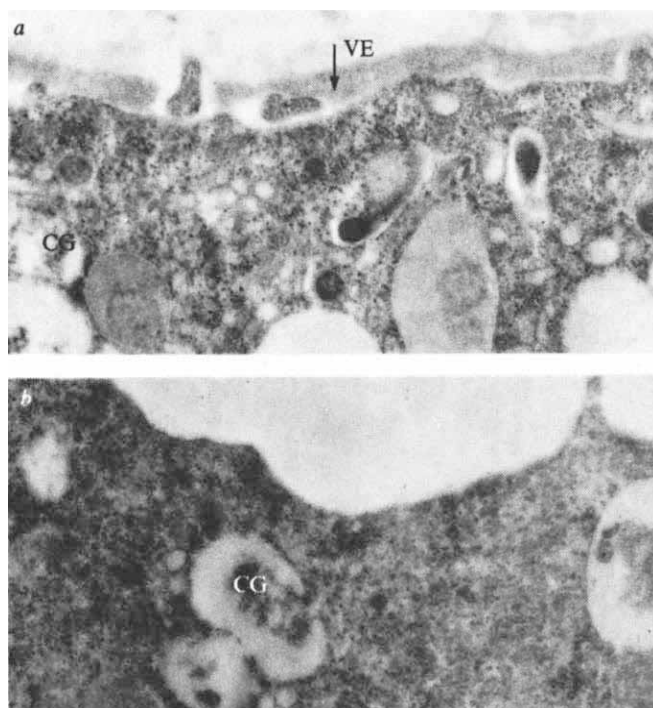
Test samples	Oocyte maturation (%) mean $\pm$ s.e.
(A)	
α -(1 $\rightarrow$ 6)-Mannan	100 $\pm$ 0
Glycogen from rabbit liver	97.0 $\pm$ 1.3
Dextran T 40 (MW, 40,000)	98.0 $\pm$ 1.8
(B)	
D-Galactose	100 $\pm$ 0
D-Mannose	95.8 $\pm$ 3.4
L-Fucose	95.7 $\pm$ 2.3
(C)	
α -(1 $\rightarrow$ 6)-Heterogalactan	0 $\pm$ 0
α -(1 $\rightarrow$ 6)-Galactobiose	3.4 $\pm$ 1.6
α -(1 $\rightarrow$ 6)-Galactotriose	1.9 $\pm$ 1.6
α -(1 $\rightarrow$ 6)-Galactotetraose	0 $\pm$ 0
(D)	
Maltose $\begin{matrix} 1 & 4 \\ \text{G} \longrightarrow & \text{G} \end{matrix}$	100 $\pm$ 0
Maltotriose $\begin{matrix} 1 & 4 & 1 & 4 \\ \text{G} \longrightarrow & \text{G} \longrightarrow & \text{G} \end{matrix}$	98.9 $\pm$ 0.9
Maltotetraose $\begin{matrix} 1 & 4 & 1 & 4 & 1 & 4 \\ \text{G} \longrightarrow & \text{G} \longrightarrow & \text{G} \longrightarrow & \text{G} \end{matrix}$	100 $\pm$ 0
Isomaltose $\begin{matrix} 1 & 6 \\ \text{G} \longrightarrow & \text{G} \end{matrix}$	100 $\pm$ 0
Isomaltotriose $\begin{matrix} 1 & 6 & 1 & 6 \\ \text{G} \longrightarrow & \text{G} \longrightarrow & \text{G} \end{matrix}$	100 $\pm$ 0

In each experiment, the minimum concentration of 1-MA for inducing 100% oocyte maturation was added. This was the most suitable concentration for detecting the inhibitory effect. Concentration of test samples was 0.83%.

α -(1 $\rightarrow$ 6)-Mannan was provided by Dr T. Nakajima, Tohoku University.

G, Glucose; MW, molecular weight.

Fig. 3 Electron micrographs of the oocytes of *Asterina pectinifera*. *a*, Normal cell surface of the oocytes. The surface is covered with the continuous vitelline envelope (VE). CG, Cortical granule ($\times 22,050$). *b*, Surface of the trypsinised oocyte. The vitelline envelope is almost removed by trypsinisation for 4 h at 24 $^{\circ}\text{C}$ with 1% trypsin solution (Difco, 1:250) dissolved in HASW ($\times 22,050$).



were also ineffective at the same concentration. α -(1 $\rightarrow$ 6)-Galactobiose, α -(1 $\rightarrow$ 6)-galactotriose and α -(1 $\rightarrow$ 6)-galactotetraose, obtained from the partial hydrolysate of α -(1 $\rightarrow$ 6)-HGaln³, were inhibitory (Table 1). The minimum concentration of 1-MA that induced 100% maturation was $30 \times (5.2 \times 10^{-6} \text{ mM})$ in the presence of 0.83% (FC) α -(1 $\rightarrow$ 6)-galactotetraose (Fig. 1). No gluco-oligosaccharides had any inhibitory effect, even if the binding form was α -(1 $\rightarrow$ 6)-linked types as the galactosides. Thus the inhibition was due to phenomena specific to α -(1 $\rightarrow$ 6)-galactan or α -(1 $\rightarrow$ 6)-galacto-oligosaccharides.

We then considered whether the inhibitor might bind directly to 1-MA in the extracellular space, or in the cytoplasm after penetrating the membrane, or on the cell surface. The first possibility was excluded because when $192 \times (5.2 \times 10^{-6} \text{ mM})$ 1-MA was mixed with an equal volume of 5% α -(1 $\rightarrow$ 6)-HGaln for about 1 h at 0–4 °C, and 10 μ l of this mixture was added to 20 μ l of starfish oocyte suspension in HASW, the extent of inhibition was 64.4% compared with 100% with the same concentration of 1-MA (FC, $32 \times (5.2 \times 10^{-6} \text{ mM})$) and α -(1 $\rightarrow$ 6)-HGaln (FC, 0.83%) after preincubation of oocytes with α -(1 $\rightarrow$ 6)-HGaln.

This was supported by chromatography, using α -(1 $\rightarrow$ 6)-galactotetraose because α -(1 $\rightarrow$ 6)-HGaln showed trace absorption at 260 nm. A mixture of 0.4 ml of 5% α -(1 $\rightarrow$ 6)-galactotetraose, 0.4 ml of 1-MA solution and 0.8 ml of HASW was stored at 4 °C for 3 h and adapted for a Sephadex G-25 column (2.64 $\times$ 85 cm). Elution was carried out with a 2/3 concentration of HASW. Fractions (5 ml) were collected at a flow rate of 10 ml h⁻¹. 1-MA and α -(1 $\rightarrow$ 6)-HGaln were completely separated by the chromatography (Fig. 2).

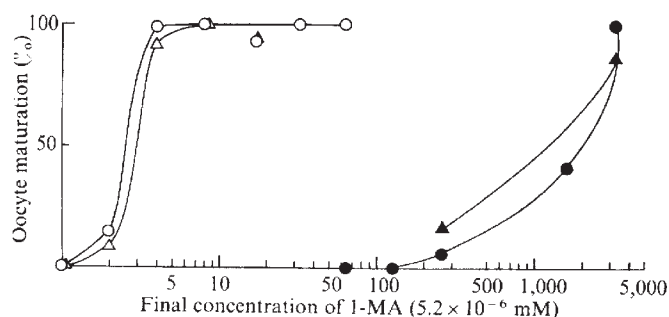


Fig. 4 Dose-response curve of the trypsinised oocytes to 1-MA and α -(1 $\rightarrow$ 6)-HGaln. The trypsinisation was carried with 1% trypsin solution for 4 h at 24 °C. $\circ$, Untreated oocyte, no α -(1 $\rightarrow$ 6)-HGaln; $\bullet$, untreated oocyte, + α -(1 $\rightarrow$ 6)-HGaln; $\triangle$, trypsinised oocyte, no α -(1 $\rightarrow$ 6)-HGaln; $\blacktriangle$, trypsinised oocyte, + α -(1 $\rightarrow$ 6)-HGaln. The trypsinised oocytes showed the same degree of response for 1-MA and α -(1 $\rightarrow$ 6)-HGaln as the untreated oocytes.

As the S value of α -(1 $\rightarrow$ 6)-HGaln is 1.55, it is unlikely that it penetrates the cell membrane. This was confirmed as follows. After pretreatment of oocytes with 0.83% α -(1 $\rightarrow$ 6)-HGaln for 30 min at 0–4 °C, they were washed twice with HASW, and their sensitivity to 1-MA was examined. The minimum concentration that induced 100% maturation was about $15 \times (5.2 \times 10^{-6} \text{ mM})$ (Fig. 1), far less than the value obtained in the presence of exogenous α -(1 $\rightarrow$ 6)-HGaln. Some of the inhibitory effect, however, remained, even after washing. This suggests that the receptor of α -(1 $\rightarrow$ 6)-HGaln is present on the cell surface.

To locate the receptors of α -(1 $\rightarrow$ 6)-HGaln and 1-MA, the vitelline envelope was almost removed from oocytes by incubation for 4 h at 24 °C with 1% trypsin dissolved in HASW (Fig. 3b). Then oocytes showed the same degree of sensitivity to 1-MA and the same degree of inhibition as untreated oocytes (Fig. 4). The receptor of 1-MA and α -(1 $\rightarrow$ 6)-HGaln may therefore be on the plasmalemma.

We thank Professor K. Matsuda for providing gluco-oligosaccharides.

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Production of ¹⁴C- and ¹¹C-labelled biomolecules using ionised gases

To establish a rapid method for eventual use in incorporating ¹¹C into important biomolecules, we have studied the interaction of ionised gases with a number of natural products. Our findings on the degradation of cholesterol induced by ionised gases¹ led us to anticipate that such labelling might occur if the gas contained a radioactive label and we present results with ¹⁴CO which verify the feasibility of the method. The same approach could also be used with ¹¹C, a positron-emitter which is too short lived for most conventional organic chemical labelling procedures². The resulting tracer biomolecules should have widespread applications in chemistry, biochemistry, biology and nuclear medicine.

The methods and apparatus are broadly similar to those already described¹. In brief, the principle is as follows (Fig. 1): ¹⁴CO gas is introduced into a vacuum system where it is bombarded by a 190-eV electron beam (E) to yield³ ¹⁴CO⁺, ¹⁴C⁺, O⁺, atomic ¹⁴C and O, and excited ¹⁴CO.

Fig. 1 Principle of the labelling method. Electrons emitted by a filament, F, are collimated by a magnetic field, B, to form an axial electron beam E, which is accelerated towards the cylinder C by the potential V_c and collected on a plate P on further acceleration by the potential V_p . Inside the cylinder C, the electrons collide with ¹⁴CO molecules (O) and produce an ionised gas containing atoms, ions and excited molecules. These highly reactive species drift to the inner surface of the cylinder C which is coated with a thin organic film. The reactions occurring on the surface produce ¹⁴C-labelled compounds which are analysed by chromatography and crystallisation to constant specific activity.

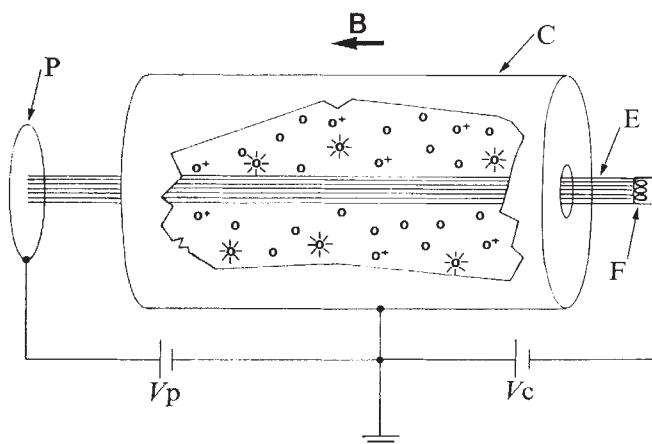
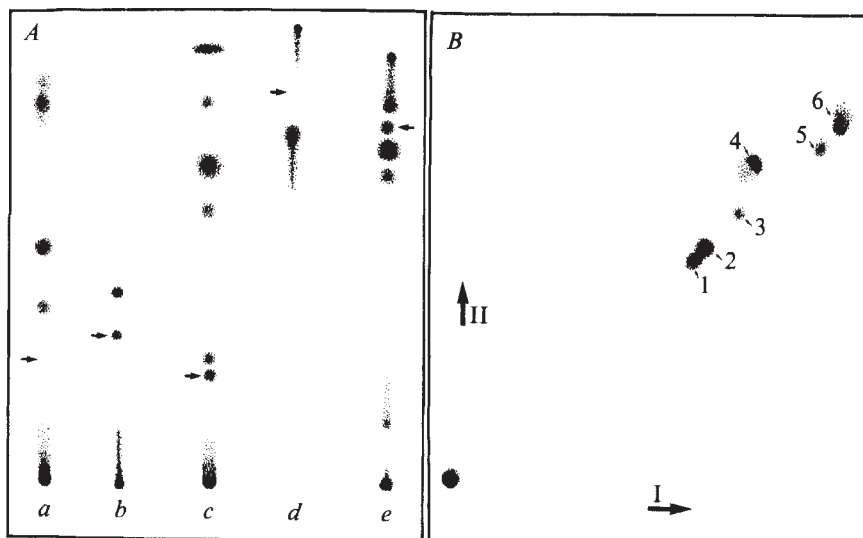


Fig. 2 Tracing of an autoradiogram after thin-layer chromatography of the ^{14}C products obtained from the interaction of organic substrates with ^{14}CO ionised gas. **A**, The position of the parent compound which was added as a marker is indicated by an arrow in each case. The starting materials were: *a*, oestradiol; *b*, 5,6-dimethylbenzimidazole; *c*, D-arabinose; *d*, thymine; *e*, glycine. Chromatographic systems were: *a*, benzene-ethyl acetate (3:2); *b*, *c*, methanol-chloroform (4:1); *e*, methanol-benzene (2:3), all on silica gel; and *d*, *n*-butanol-acetic acid-water (80:12:30), on cellulose. **B**, Two dimensional analysis of cholesterol after exposure to ^{14}CO ionised gas. Chromatographic solvents were: I, hexane-ethyl acetate (3:2) and II, chloroform-acetone (95:5). Most of the total activity was recovered in spots 2, 4 and 6. By placing reference compounds with the labelled material before analysis, spots 2 and 4 were found to coincide with cholesterol and cholesterol formate, respectively. The identity of the ^{14}C -cholesterol fraction was confirmed by crystallisation to constant specific activity.



These species drift to the inner surface of the cylinder (C) which is coated with organic material. Reactions with the substrate yield a number of labelled products, as shown by chromatographic analysis (Fig. 2A). Steroids, proteins, sugars and a number of heterocyclic compounds were treated thus and gave radioactive products in varying yields, some of which migrated with the starting material. Typically, a 2-min bombardment of 10–100 μg of substrate with carrier-free ^{14}CO gave a total yield of labelled products of up to 1 mCi mmol $^{-1}$. Figure 2A shows autoradiograms obtained from a one-dimensional thin-layer chromatographic analysis of experiments with oestradiol, thymine, 5,6-dimethylbenzimidazole, D-arabinose and glycine. A similar analysis was carried out in two dimensions for cholesterol (Fig. 2B) and reveals the presence of six major labelled compounds. Two of these (2 and 4) migrate at the positions of cholesterol and cholesterol formate, respectively. The identity of compound 2 (Fig. 2B) as the labelled, but otherwise unaltered, parent molecule was established by crystallisation to constant specific activity of the free sterol and of its acetate derivative (Table 1). ^{14}C -cholesterol yields of up to 0.1 mCi mmol $^{-1}$ were demonstrated in this manner. Although this value may seem low compared with chemical

labelling procedures, the replacement of ^{14}C by ^{11}C with its 10 8 -fold shorter lifetime, would increase the activity to as much as 10 Ci μmol^{-1} . Even higher yields can be obtained for some of the secondary products of other substrates. This method thus seems particularly promising for the application of ^{11}C in bioorganic sciences and nuclear medicine.

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Table 1 Crystallisation of ^{14}C -cholesterol from the interaction of cholesterol with an ionised ^{14}CO gas

Crystallisation No.	Solvent	Specific activity (d.p.m. g $^{-1}$)
1	Methanol	13,840
2	Methanol	9,964
3	Methanol	9,341
4	Methanol	9,022
5	Petroleum ether-ethyl ether	8,064
6	Petroleum ether-ethyl ether	7,983
7	Petroleum ether-ethyl ether	8,075
8	Petroleum ether-ethyl ether	7,915
9	Methanol-ethyl ether (3-acetate)	8,131
10	Methanol-ethyl ether (3-acetate)	7,974
11	Methanol-ethyl ether (3-acetate)	8,038

Cholesterol (60 μg) was bombarded as described in the text. Labelled products were recovered from the cylinder C (Fig. 1) with ethyl acetate and diluted with 4.87 g of carrier cholesterol. After the eighth crystallisation, the remaining cholesterol was converted to the 3-acetate and the specific activities were adjusted to the equivalent weight of cholesterol.

Erratum

In the article "Antiviral and cell growth inhibitory activities reside in the same glycoprotein of human fibroblast interferon" by E. Knight, Jr (*Nature*, 262, 302; 1976) the first line of column 1, page 303 should read . . . was exercised when observing growth inhibition with im-. . . and not as printed.

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reviews

Economy and ecology

Peter D. Moore

The Economy of Nature: A Textbook in Basic Ecology. By Robert E. Ricklefs. Pp. v+455. (Chiron: Portland, Oregon, January 1976.) \$13.95. *Methods in Plant Ecology.* Edited by S. B. Chapman. Pp. viii+536. (Blackwell Scientific: Oxford and London; Halsted: New York, 1976.) Cloth £15; paper £9.75.

PUBLISHERS of ecological textbooks would do well to consider Gause's principle of competitive exclusion. The cash in a student's pocket is a distinctly limited resource for which textbooks must compete; any new text, to be successful, must carve out a niche for itself in what is already a fairly saturated market. And in so doing it will inevitably displace other texts from that volume of literary hyperspace which they currently occupy. When considering a new text, one must therefore assess its competitive ability in the niche for which it has been designed.

Ricklef's new book is aimed at bridging the gap between the voluminous texts which seek to cover the whole gamut of ecological thought and the brief, sometimes superficial, introductory books. It has a broad coverage, following a very similar sequence to his previous book (*Ecology*, Thomas Nelson, 1973), but the text penetrates far less deeply than that volume. The style is light and readable and is uninterrupted by references to original papers. Some new illustrations, both verbal and pictorial, are introduced, although many of the old ones are retained. There is still a strong emphasis upon zoological and North American examples of the principles described.

Is there a niche for this textbook in the current market? I do not consider it a serious competitor with the variety of good textbooks currently available (including Ricklefs' *Ecology* and also Colinvaux, *Introduction to Ecology*, Wiley, 1973; *Ecology*, Krebs, Harper and Row, 1972; *General Ecology*, McNaughton and Wolf; Holt, Rinehart and Winston, 1973) for that portion of the 'resource' provided by British University students of biology. The low level of numerical treatment and the lack of references will prob-

ably cause it to fail in this respect. It is not a textbook which generates a critical approach to the data and the conclusions presented; therefore, I do not think that it will displace any weightier text from this portion of its niche. It may appeal to the broader-based groups of students possibly proceeding along paths encompassing humanities and environmental studies. Here its neglect of the numerical approach may be an asset. In this narrower niche, however, is the resource adequate to support a large population—that is how big is this specialised market? Where competition is weak it is often, although not invariably, due to low resource availability.

The second book, edited by Chapman, is making an overt bid to fill a specialised, relatively narrow niche in the textbook community, that of methodology in plant ecology. A wide range of disciplines, and methods within these disciplines is covered and, since each has a separate author, one might expect a degree of patchiness. The chapter on history of vegetation covers an enormous range of ideas, methods and techniques, but never in sufficient depth to provide an adequate laboratory manual. The key to pollen grains is inadequate, inaccurate and misleading. The description of vegetation is dealt with far too briefly, in the following chapter. Several good textbooks are now available on this subject and this short survey adds little new.

The chapter on production ecology is useful in that it explains very clearly and precisely the basis of productivity estimation and the various growth formulae that can be used. Once again it provides background information and methodological appraisal, but space (presumably) does not allow the detailed descriptions of techniques which would be required by neophytes.

Other topics covered include physiological ecology, soils, climatology, chemical analysis and data collection systems. In almost all cases the sheer pressure of space results in the abbreviation of technique description, although references to the literature are always numerous. The imposed brevity, however, does mean that the serious student of any specialised discipline is likely to look elsewhere for information, often to a text specifically on the required techniques. Competitive displacement is therefore an unlikely outcome of this publication. There remains the question of whether the gathering of all these varied techniques under a single cover might provide the book with a distinct niche of its own. I fear that this is unlikely; it may find its way into libraries or on to a few private shelves, but I cannot envisage its being bought by students; and this is where the real resource lies. □

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Pollution control

The Estimation of Pollution Damage. (Studies in Environmental Pollution.) By P. J. W. Saunders. Pp. 126. (Manchester University: Manchester, April 1976.) £6.95.

NO-ONE approves of pollution. Unfortunately, however, its control is not simple. A great deal of effort and money may be devoted to reducing the amount of some toxic substance in our environment, with little or no useful effect, because preliminary studies have not been made to find out what, if any, harmful effects the

substance is really producing. Our resources are clearly insufficient to deal with every possible source of pollution, and it is therefore essential that we discover which are the greatest danger, and concentrate, first, on their control.

In this book Dr P. J. W. Saunders of the Natural Environment Research Council, and formerly of the Pollution Research Unit at Manchester University, sets out to show how the real, harmful effects of pollution may be measured, so that effective control measures may be planned. The book

is primarily intended for students and administrators concerned with this subject, although it can also be recommended to the more studious members of the wider public who are often so vocal about environmental problems.

Dr Saunders defines pollution as "the introduction by man of waste matter or surplus energy into the environment which directly or indirectly causes damage to man and his environment, *other than himself*, his household, those in his employment or those with whom he has a direct trading relationship". I am unable to understand why self-inflicted, domestic and occupational exposures are excluded except where they interact with other types of pollution. It may be more difficult to evaluate this type of damage in cash terms but they

are equally serious and equally necessary to control.

The amount of information condensed into this small book is quite remarkable. The whole field of air, water, soil and of estuaries, and the specific problems caused by persistent pollutants is summarised in under 20 pages. Global problems are dealt with in about the same space. The remainder of the text describes the many techniques involved in estimating and measuring pollution, with a number of well-chosen practical examples. The extreme compression does not make for easy reading, which may discourage less-informed readers. Students who often only receive a superficial introduction to this subject will benefit most from its content.

Kenneth Mellanby

Changing spectrum for crop plants

Evolution of Crop Plants. Edited by N. W. Simmonds. Pp. xii+339. (Longman: London and New York, 1976.) £14.00.

THIS is an excellent book in which the title is used in its widest sense to convey the whole spectrum of a crop's development from origin to modern varieties, and its present and future potential role in the changing demands of world agriculture. Not since De Candolle has a book attempted to cover all the major and many minor crop plants grown in the world today; this book should therefore prove invaluable to all those involved internationally in crop improvement programmes.

The book is multi-authored, with 86 chapters detailing individual crops, arranged alphabetically by family, with the last two covering timber trees and minor crops. Each has an introduction and four sections on cytotaxonomic background, early and recent history and prospects, ending with a bibliography listing key and source references. Chapters range from 2,000–6,000 words depending on agricultural importance and depth of evolutionary understanding.

The absolute merit of individual chapters must be judged by the relevant crop specialist. All combine a range of scientific disciplines in framing a crop's history in a concise and comprehensive manner. In some instances emphasis on the former has produced some formidable terminology in the cytotaxonomic section (for example, Papaya, ch 8, and Fig, ch 59) and early history section (in

describing early plant migrations); and simplification or additional explanation would be helpful, although in both the regular use of diagrams aids presentation. The recent history and prospects sections detail the application of modern breeding methods in variety development, and attempt to identify the inherent problems for the future. In some cases, however, little mention is made of the local importance of a crop outside its main production area, where different though equally important problems may occur—two examples being cotton and groundnuts in Africa (south of the Sahara).

Of particular note for the future is the recurring theme in many chapters, on crops with a long breeding history, of the poor management in the past of crop genetic resources, resulting in depletion of available genetic variability, which may limit future advances. That this should not be repeated is stressed by a number of authors describing crops which have recently received attention and which, although only now grown in subsistence agriculture, are likely to play a key role in the future in improving living standards in developing countries. It is also evident that there are crops, such as cucurbits and edible aroids, whose potential for production in marginal tropical areas is recognised but as yet little exploited.

I can only agree with the editor's words that the book "concentrates on the particular and, in doing so reveals . . . , how insecure our knowledge often is, how much more work is needed and how often, even now, the right questions have not yet been asked".

H. E. Gridley

H. E. Gridley is a research assistant in the department of applied biology at the University of Cambridge, UK.

Foraminifera

Recent Foraminifera. By Esteban Boltovskoy and Ramil Wright. 515 pp. (Junk, The Hague, 1976.) 125 DF1.

A THOROUGH revision and updating of Boltovskoy's original *Los Foraminiferos Recientes* (Buenos Aires, 1965) has resulted in a comprehensive new text, furnished with well chosen and clearly reproduced figures, a carefully compiled bibliography and a good index, the whole being attractively printed on good paper and sturdily bound. The authors deserve high praise for so successfully summarising such a wide range of information, for always emphasising the significance and practical value of each aspect of the subject, and in producing such a readable book. It can be read from cover to cover with pleasure, or simply dipped into with profit.

No previous knowledge of the foraminifera is assumed, yet the authors quickly introduce the reader to the results of research which were very new when their manuscript was completed (January, 1975). Foraminiferal morphology and biology are clearly and concisely described, the scientific significance of foraminiferal studies is made plain, and the distribution and ecology of the foraminifera are described and discussed in their oceanographic, biostratigraphic and palaeo-oceanographic contexts. Taxonomy, systematics and problems of synonymy are dealt with concisely and realistically. There are most useful chapters covering techniques of collection, preparation, culture and storage, which the authors have tried and tested.

This book is no mere library compilation of other scientists' publications: rather, it is the result of long experience of practical work with foraminifera by the authors themselves. The book is intended for workers of all kinds, from the beginning student to the academic and industrial researcher, for those who have access to large research budgets and to those who can lay hands on only the most simple equipment and most modest resources. The authors succeed admirably.

Of course, there are the inevitable minor errors (a dreadfully confused Fig. 117 is, perhaps, the worst). The book should, however, be compulsory reading for all students of the foraminifera, be they protozoologists or micropalaeontologists, biological oceanographers or biogeographers.

F. T. Banner

Dr Banner is a reader in the Department of Oceanography at University College, Swansea, UK.

Understanding invertebrates

A Functional Anatomy of Invertebrates. By V. Fretter and A. Graham. Pp. vi+589. (Academic: London and New York, March 1976.) £12.50; \$31.

THIS is a book meant to be read. It is possible to do so from one end to the other by those who know something about invertebrates; and the experience is refreshing. But when I finished it I wanted to write a different book about invertebrates. This somewhat ungracious comment is a compliment in a way, for those of us who know the authors' many contributions to our understanding of invertebrates will be neither surprised nor disappointed by this book.

It is not possible in so short a space to argue about what is meant by 'functional anatomy' for rather like the words 'physiology' and 'biology', it is interpreted in different ways by different people. So this is a peculiarly individual book and we must be grateful to them for so elegantly setting down their approach after a life-time's experience of teaching and research, and to Academic Press for producing it so well.

How fortunate students are; with Barnes, Meglitsch, and Marshall and Williams as basic texts, and with Barrington, and now this new book, it is possible to obtain a far better understanding of the invertebrates than ever before. I must emphasise the inherent readability of this new book, for so many basic textbooks are so packed with detail that they serve more for reference, or at least can be read with pleasure only in parts.

Fretter and Graham have given us an account of all the more important invertebrates (but excluding protochordates); although exemplary in style and clarity, the illustrations are not numerous and serve to illustrate the text. This is a Koran for the converted and in my opinion it will be appreciated most by those whose interest has already been caught.

How wise it was of Sinauer to put the stereoscan picture of *Didinium* attacking *Paramoecium* on the cover of E. O. Wilson's *Life on Earth*. How much more exciting it would have been in the present case to have ultrastructural details of the coelenterate nematocyst for example, or a stereoscan view of the choanocyte. What a pity there is so little on parasitic protozoa with no life cycle diagrams. Only seventeen pages on land arthropods. No illustration (even) of the cephalopod eye or the octopus brain.

I must confess that if I had just left

the sixth-form having used Roberts, Berrill, and Clegg I would think this book, on first impression, was dull, but I would appreciate it more I think, when I had learnt more about the invertebrates. For there is no 'ideal' book to cover so wide a field; there can be no single volume which can provide all a student needs, especially when most of us devote less of the degree course to such basic zoology.

Published at a price most students will be reluctant to pay, it is nevertheless a book no Department can afford to be without. It is a book which provides a stimulating comparison with Barrington and others, and which, I would predict, will be appreciated more and more by students as they come to know the invertebrates better.

R. P. Dales

R. P. Dales is Professor of Zoology at Bedford College, University of London, UK.

Caribbean-Mexican tectonics

The Ocean Basins and Margins. Vol. 3: The Gulf of Mexico and the Caribbean. Edited by A. E. M. Nairn and F. G. Stehli. (Plenum: New York, 1975.)

THIS is the third in a series intended to document the world's ocean basins and their margins. Previous volumes have covered the North and South Atlantic Oceans.

The Gulf of Mexico and the Caribbean Sea have been the subject of many geological and geophysical studies that have added to the wealth of data obtained by the oil industry in the shelf and onshore areas. Not surprisingly, however, there is no firm consensus on the tectonic evolution of the area, although two views are held. The mobilist view seeks to explain the evolution of the Caribbean by seafloor spreading and plate tectonics, whereas the stabilists believe that the Caribbean Sea is an ancient oceanic structure.

There are fifteen chapters in the book. The introductory chapter by Uchupi reviews the Physiography of the Gulf of Mexico and Caribbean Sea. The two succeeding and particularly good chapters discuss Geophysical Studies in the Gulf of Mexico (Martin and Case) and Geophysical Studies in the Caribbean Sea. Fox and Heezen present valuable rock dredge data, and seismic refraction and reflection studies in the Geology of the Caribbean Crust. Other chapters discuss various aspects of Palaeozoic geology around the Gulf of Mexico and Caribbean Sea. There

are detailed reviews of key subjects such as the Northern Termination of the Andes and the Palaeozoic and Mesozoic tectonic belts of Mexico and Central America. Areas discussed in more detail are Cuba, Hispaniola, Jamaica and the Nicaraguan Rise. The Lesser Antilles and Aves Ridge are reviewed by J. F. Tomblin with emphasis on the volcanology, petrology and geochemistry of the arc. T. W. Donnelly concludes the book with a review of critical problems in understanding the Gulf of Mexico and Caribbean Sea.

Like the preceding volumes, this book has its deficiencies. Some of the chapters are little more than dry structural and stratigraphic descriptions of particular areas with little comment on the wider implications. Time has invalidated earlier conclusions—in one case beds previously considered as Permian are now known to be Late Cretaceous. In spite of this criticism, the chapters will be valuable as source material.

A more serious criticism of the book is the absence of any single detailed discussion of the marine geology of the Barbados Ridge and Lesser Antilles arc. Reportedly, wells on Barbados show evidence of imbrication, of obvious relevance in subduction. The Deep-sea Drilling Project results are cursorily treated and would have merited a whole chapter.

One of the authors rightly comments that a synthesis of the land and marine data is required and another that there is a "negative correlation between Caribbean field geology experience and readiness to apply the recent concepts of seafloor spreading". A useful example is the problem posed by the Jurassic igneous rocks of La Desirade situated in the Tertiary Lesser Antilles Arc. Are these rocks *in situ* or are they a tectonically transported slice of old Atlantic ocean crust? In such areas multichannel seismic surveys may resolve such tectonic anomalies.

Multichannel seismic surveys emerge from the book as priority measures for the Gulf of Mexico and Caribbean Sea to resolve such questions as continuity of salt across the Gulf and the distribution of beds below horizon B¹¹ in the Caribbean itself. Such surveys, calibrated by deep-sea drilling, may resolve many of the problems of the stabilism-mobilism controversy, reviewed and briefly discussed by the contributors to the book.

David G. Roberts

David Roberts is a Principal Scientific Officer at the Institute of Oceanographic Sciences, Godalming, UK. He was recently Co-Chief Scientist on Leg 48 of Glomar Challenger at the Margins of Biscay and Rockall as part of the Deep-Sea Drilling Project.

announcements

Appointment

Mr E. S. Booth, CBE, MEng, FRS, Chairman of the Yorkshire Electricity Board, Leeds, will take over as President of the Institution of Electrical Engineers for 1976/77.

Awards

The Association for Radiation Research has awarded the 1976 Weiss Medal to Dr Ulrich Hagen of the Institute of Radiobiology in the Nuclear Research Centre, Karlsruhe for his work on the effects of radiation on biological molecules.

The University of Chicago has awarded the first George Willard Wheland award to Professor M. J. S. Dewar of the University of Texas for his achievement in introducing new concepts from quantum mechanics into university courses on organic chemistry.

Meetings

November 8–10, **Function and Biosynthesis of Lipids** (Sponsored by the IUB and PAABS), Sierra de la Ventana, Argentina (Dr Nicolas G. Bazan, Chicla 508,8000 Bahia Bianca, Argentina).

November 11–12, **Scientific and Technical Publishing in a Multilingual Society**, Luxembourg (Mr J. M. Gibb, DG XIII, European Centre, Kirchberg, Luxembourg).

November 22–26, **Corrosion Problems, Prevention and Practice**, Auckland, New Zealand (Conference Chairman, Australasian Corrosion Association, PO Box 5961, Wellesley Street, Auckland, New Zealand).

December 10, **New Aspects of Epithelial Transport**, London (The Secretary, Dept of Physiology, King's College, London).

December 28–31, **Coconut Research and Development**, Kasaragod, Kerala State, India (N. M. Nayar, CPCRI Regional Station, Vittal 574 243, Karnataka State, India).

January 11, 1977, **Corrosion in Agriculture**, Nottingham (B. Wilton, School of Agriculture, Sutton Bonington, Loughborough, Leics.).

January 26–28, 1977, **Electrical Properties of Biological Polymers, Water and Membranes**, New York (The Conference Director, The New York Academy of Sciences, 2 East 63rd

Person to Person

Myocrisin (sodium aurothiomalate) is said to photosensitise the skin of some patients. Any information concerning such an effect in humans or animals treated with drugs containing gold would be appreciated by Dr R. J. Wilkins, Dept. of Microbiology, University of Otago Medical School, Dunedin, New Zealand.

Smithsonian Fellowships will be awarded to support independent research in residence at the Smithsonian Institution using the collections, facilities, and laboratories and pertaining to research interests of the Smithsonian research staff (anthropology, biology, the earth sciences and the history of science and technology). Fellowships worth \$10,000 p.a. and research allowances, are granted to postdoctoral scholars, and predoctoral fellowships, of \$5,000 and research allowances, may be granted to doctoral candidates. Applications are due by January 15, 1977.

A limited number of Research Fund grants, (of ~£100) will be awarded for 1977. Application forms, together with the regulations governing the awards, may be obtained from the Education Officer, The Chemical Society, Burlington House, London W1V 0BN, and the closing date for applications is November 1, 1976.

Three awards (consisting of a silver medal and 250 guineas) for work in chemistry will be made to the British chemists who have published the most meritorious contributions in their fields of experimental chemistry. The rules for the award may be obtained from the Local Activities Officer of the Chemical Society, Burlington House, London W1V 0BN. Applications or recommendations must be received by December 31, 1976.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Street, New York, New York 10021). April 18–20, 1977, **Chemical and Molecular Lasers**, St Louis, Missouri (Deadline for abstracts: January 10, 1977) (Dr W. Q. Jeffers, Helios Inc., PO Box 2190, Boulder, Colorado 80302).

April 18–22, 1977, **Molecular Beams**, Amsterdam (A. E. de Vries, FOM-Institute, Kruislaan 407, Amsterdam, The Netherlands).

April 20–27, 1977, **First International Conference of Science Editors**, Jerusalem (Miriam Balaban, PO Box 4059, Jerusalem, Israel).

May 4–6, 1977, **Ozone Technology**, Paris (Deadline for abstracts: October 31) (International Ozone Institute, European Committee Secretariat, 52 rue d'Anjou, 75384 Cedex 08 Paris, France).

July 24–30, 1977, **40th Annual Meeting of the Meteoritical Society**, Cambridge, UK (Dr E. R. D. Scott, Department of Mineralogy and Petrology, Downing Place, Cambridge CB2 3EW).

August 15–19, 1977, **International Conference on Applied General Systems Research: Recent Developments and Trends**, Binghamton, New York (George J. Klir, School of Advanced Technology, State University of New York, Binghamton, New York 13901).

August 30–September 1, 1977, **Dopamine** (ISN satellite symposium) (Dr P. J. Roberts, Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU, UK).

September 13–16, 1977, **Unconventional Photographic Systems**, Oxford (G. W. Smith, Ozalid (UK) Ltd, Langston Road, Loughton, Essex, UK).

September 14–16, 1977, **Quantum Electronics**, Southampton (Deadline for papers: May 31) (Dr H. C. Hanna, Department of Electronics, University of Southampton, Southampton, Hants SO9 5NH).

September 28–30, 1977, **Platelets: a Multidisciplinary Approach**, Florence (Dr G. de Gaetano, Laboratory for Haemostasis and Thrombosis, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62–20157 Milano, Italy).

October 7–11, 1977, **Essential Oils**, Kyoto, Japan (Mr Yasumasa Kato, Secretary General, VII International Congress of Essential Oils, c/o Kyoto International Conference Hall, Takaraike Sakyo-ku Kyoto, 606 Japan).